Molekulární fyziologie živočichů

Růstové faktory z rodiny Wnt ve fyziologii

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&
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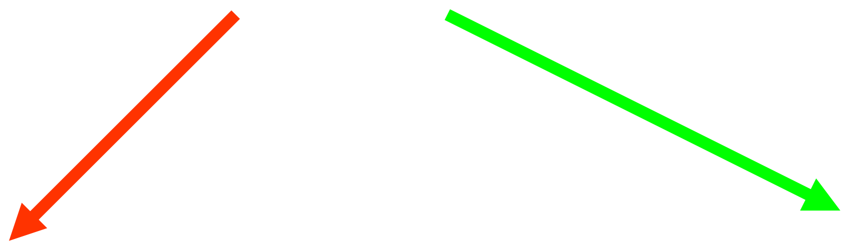
Wnts (Wingless/Int)

- family of ligands
- 19 members in human and mouse
- glycosylated and palmitoylated extracellular proteins
- short range of action, bind to extracellular matrix
- only in multicellular animals



canonical

(eg. Wnt-1 or Wnt-3a)

non-canonical

(eg. Wnt-5a)

Wnt/β-catenin dráha (= kanonická dráha)

- např. Wnt-1 nebo Wnt-3a

- induce axis duplication in Xenopus

- induce transformation of mammary cell line C57mg

-signal via nuclear translocation of β-catenin

Moon-cel2[1].swf

Příklady vývojových a fyziologických procesů regulovaných kanonickou Wnt dráhou

Maternální Wnt/β-cateninová dráha determinuje dorsální (horní) pól vyvíjející se zygoty a embrya

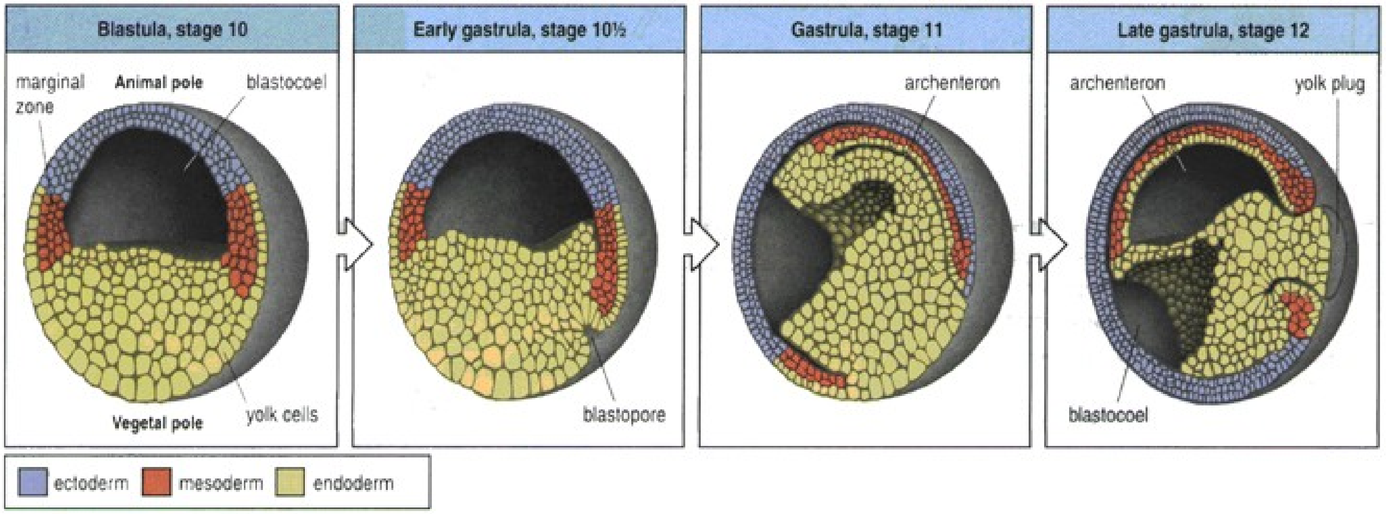
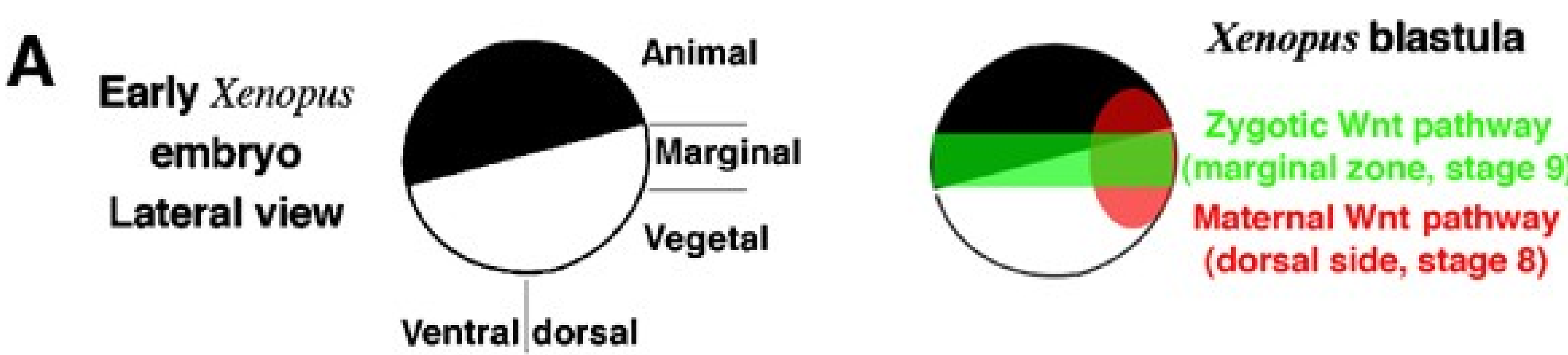
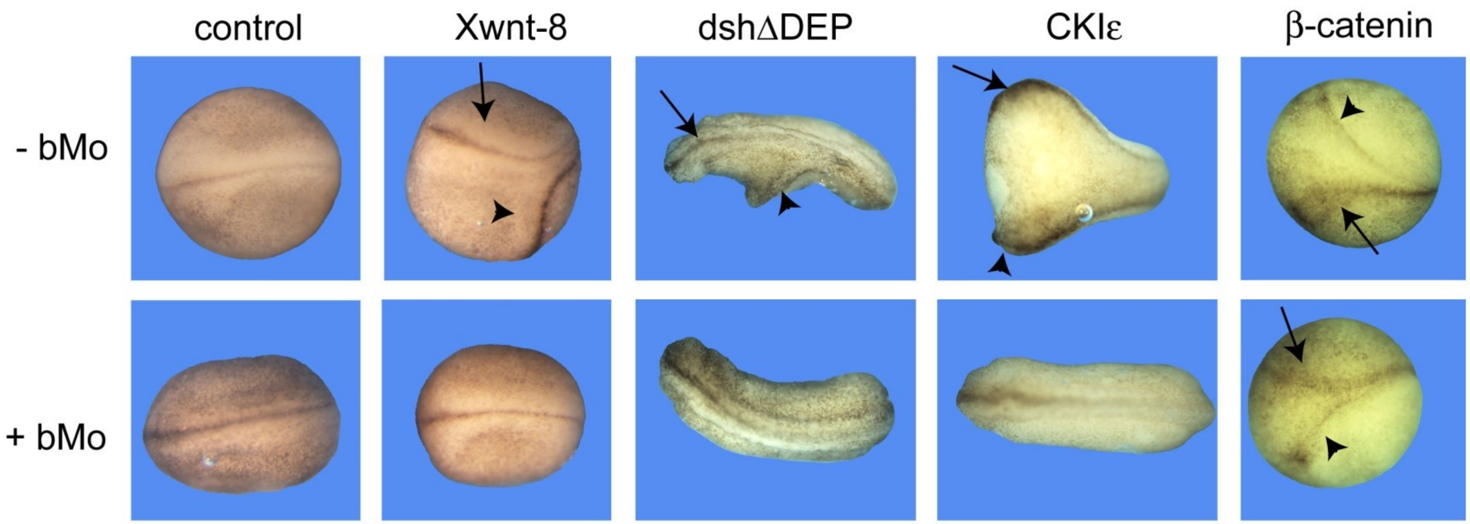


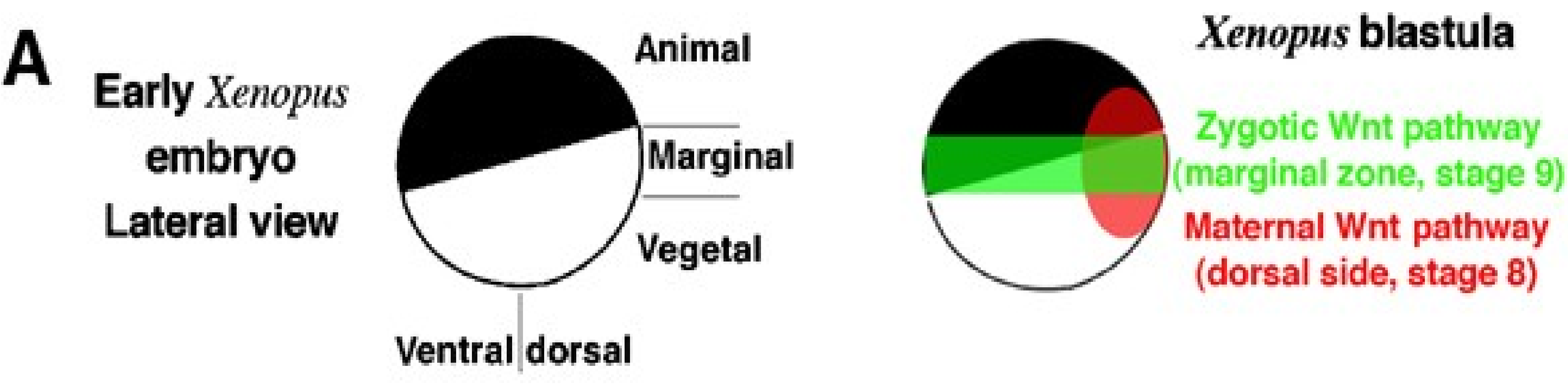
Fig. 2.6 Gastrulation in amphibians. The blastula (first panel) contains several thousand cells and there is a fluid-filled cavity, the blastocoel, beneath the cells at the animal pole. Gastrulation begins (second panel) at the blastopore, which forms on the dorsal side of the embryo. Future mesoderm and endoderm of the marginal zone move inside at this site through the dorsal lip of the blastopore, the mesoderm ending up sandwiched between the endoderm and ectoderm in the animal region (third panel). The tissue movements create a new internal cavity—the archenteron—which will become the gut. Endoderm in the ventral region also moves inside through the ventral lip of the blastopore (fourth panel) and will eventually completely line the archenteron. At the end of gastrulation the blastocoel has considerably reduced in size. After Balinsky, B.I.: 1975.



axis duplication assay:

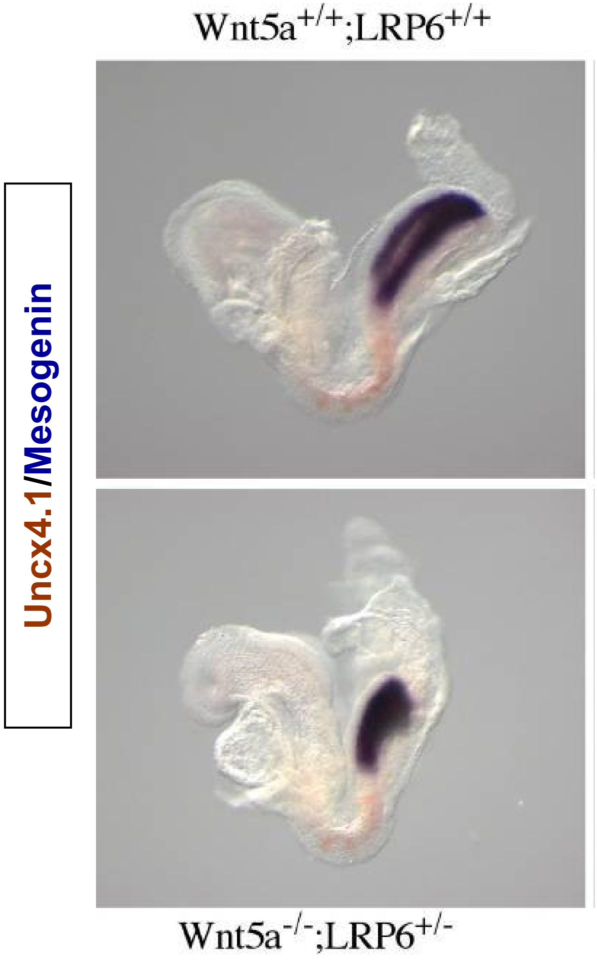


Wnt/β-cateninová dráha určuje anterioro-posteriorní (AP, předo-zadní) osu těla během gastrulace – podporuje vznik zadních a blokuje vznik předních částí těla

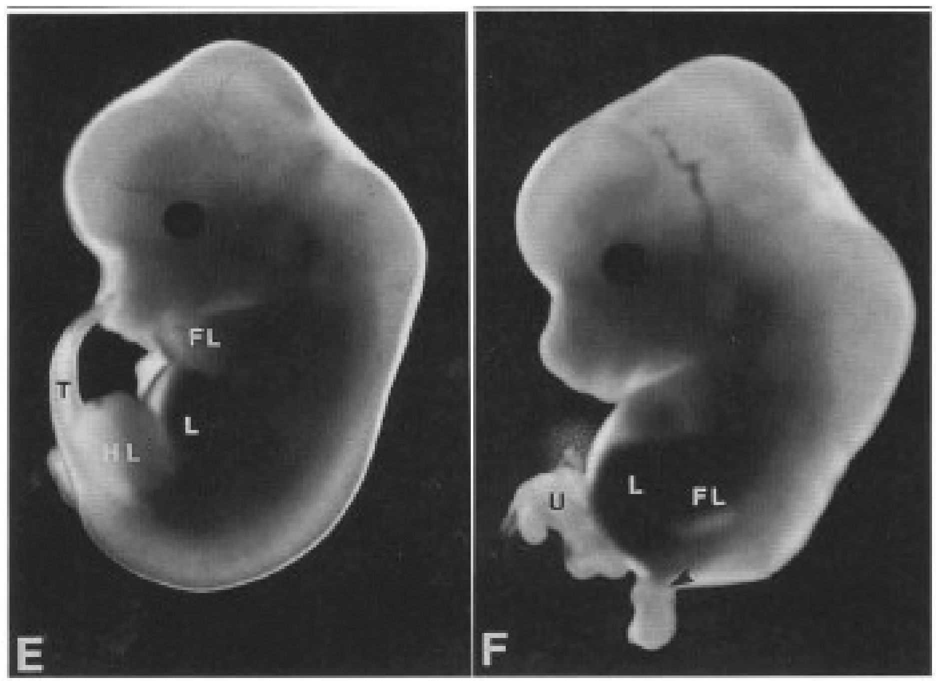


myší embryo po gastrulaci (E8.5):

Cílové geny Wnt/β-cateninové dráhy jsou exprimovány v zadní části těla.

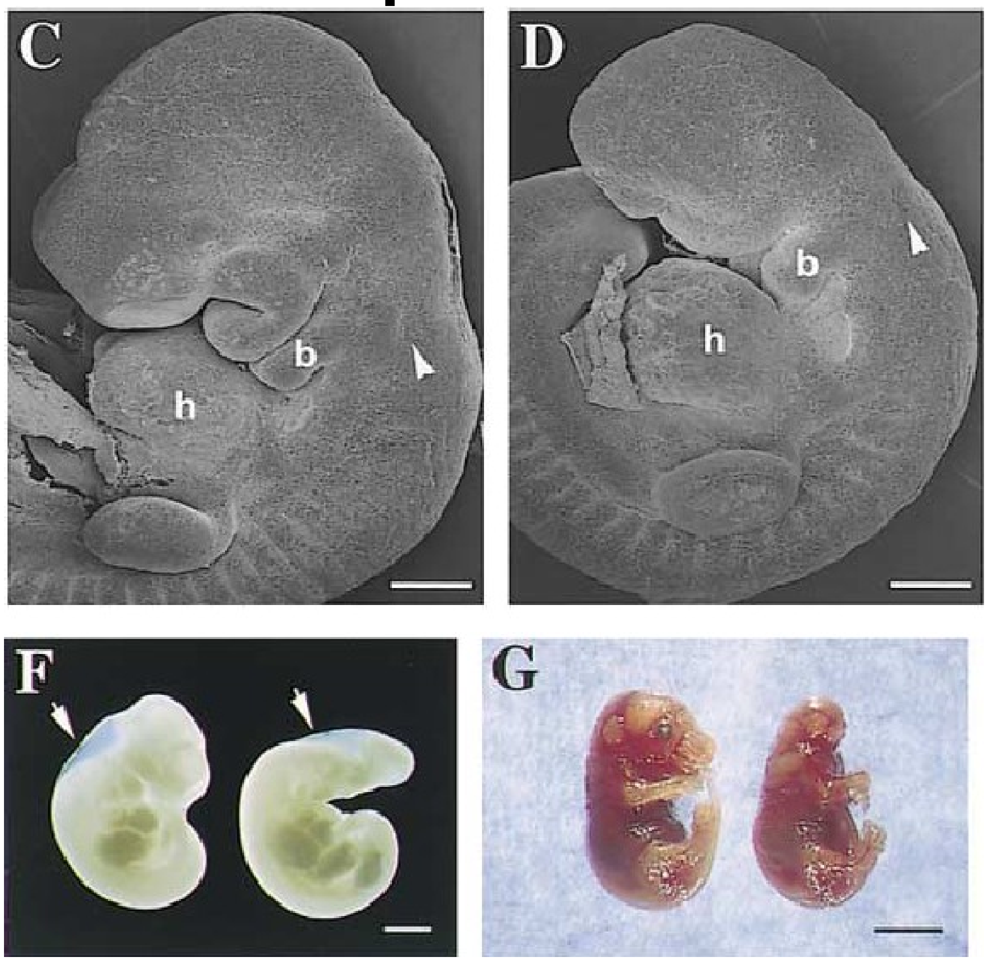


Deplece Wnt/β-kateninové dráhy při gastrulaci = ztráta zadních částí těla

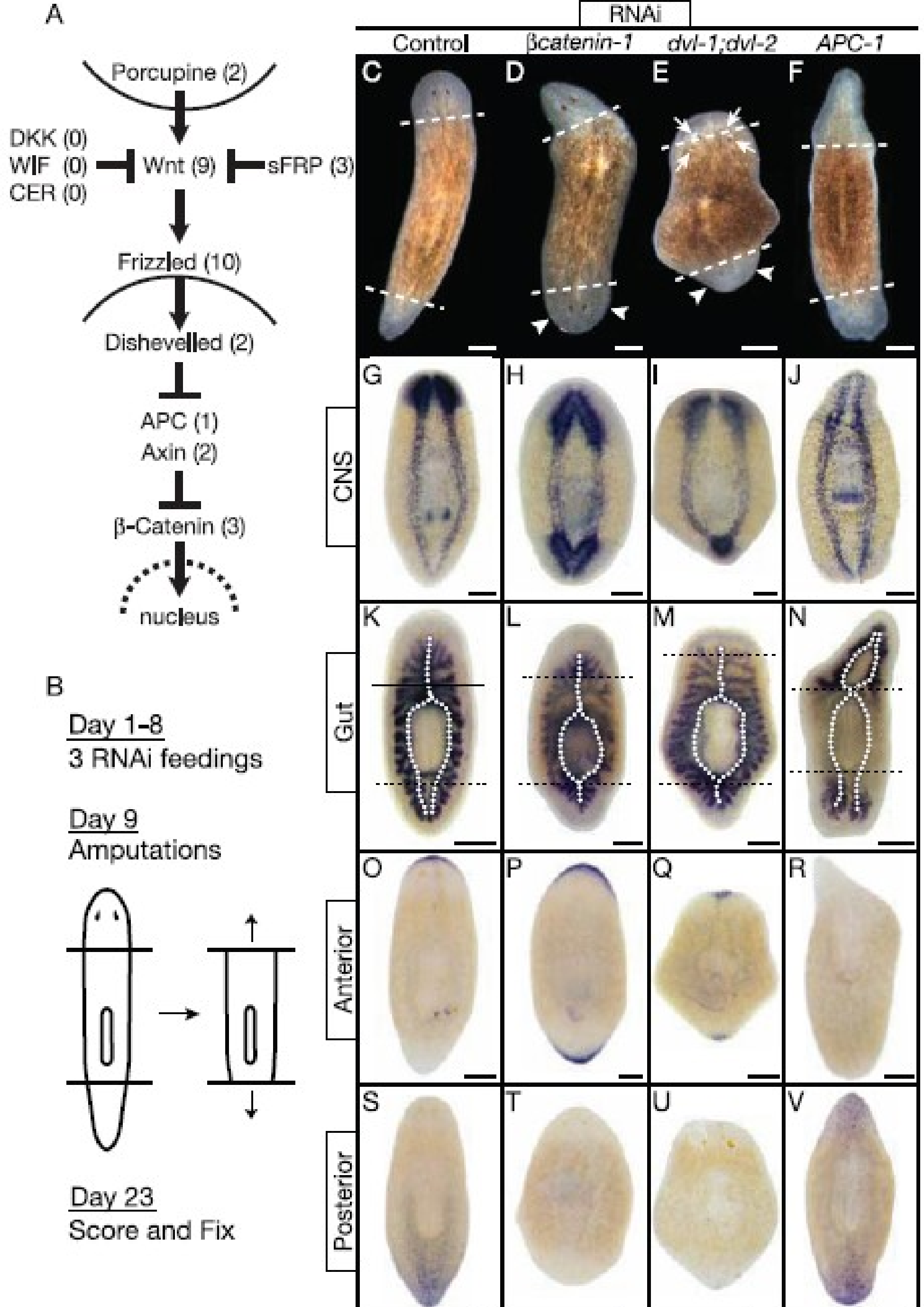


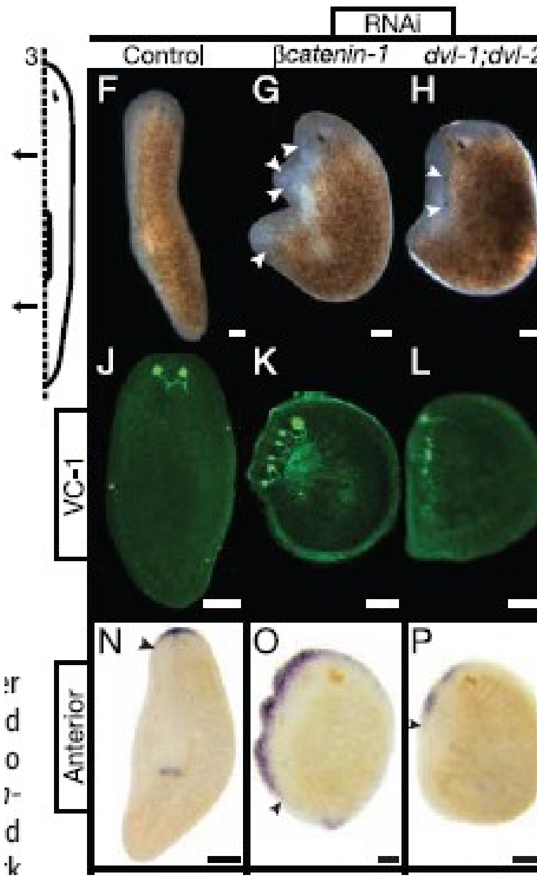
wild type Wnt-3a knockout

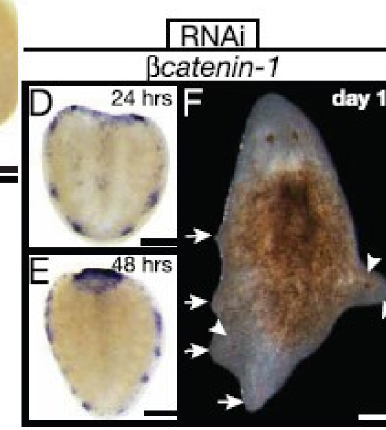
Deplece inhibitorů Wnt/β-kateninové dráhy při gastrulaci = ztráta předních částí těla



wild type vs. Dkk1 knockout







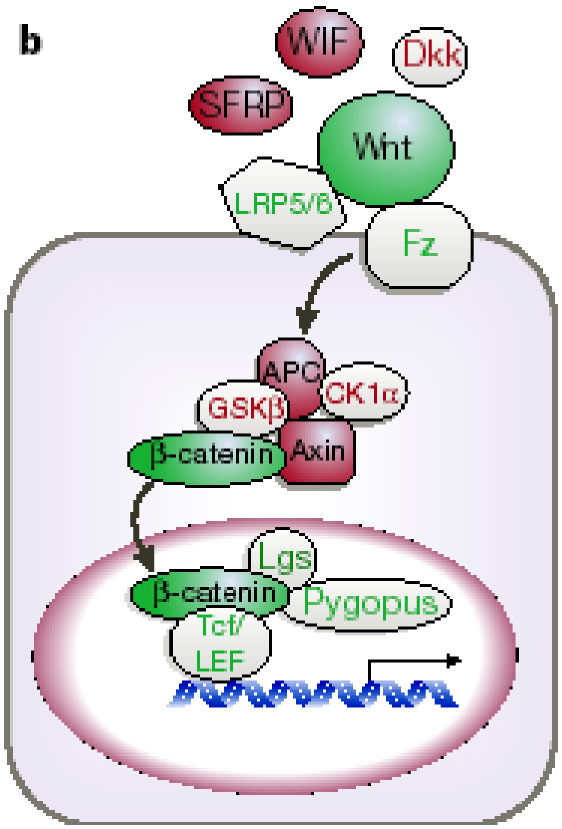
β-Catenin Defines Head Versus Tail Identity During Planarian Regeneration and Homeostasis

Kyle A. Gurley, Jochen C. Rink, Alejandro Sánchez Alvarado*

SCIENCE VOL 319 18 JANUARY 2008

Wnt/β-cateninová dráha je klíčovým regulátorem aktivace kmenových buněk jak v embryogenezi, tak v dospělých tkáních

Wnt/β-catenin dráha je velmi často deregulovaná u nádorů!



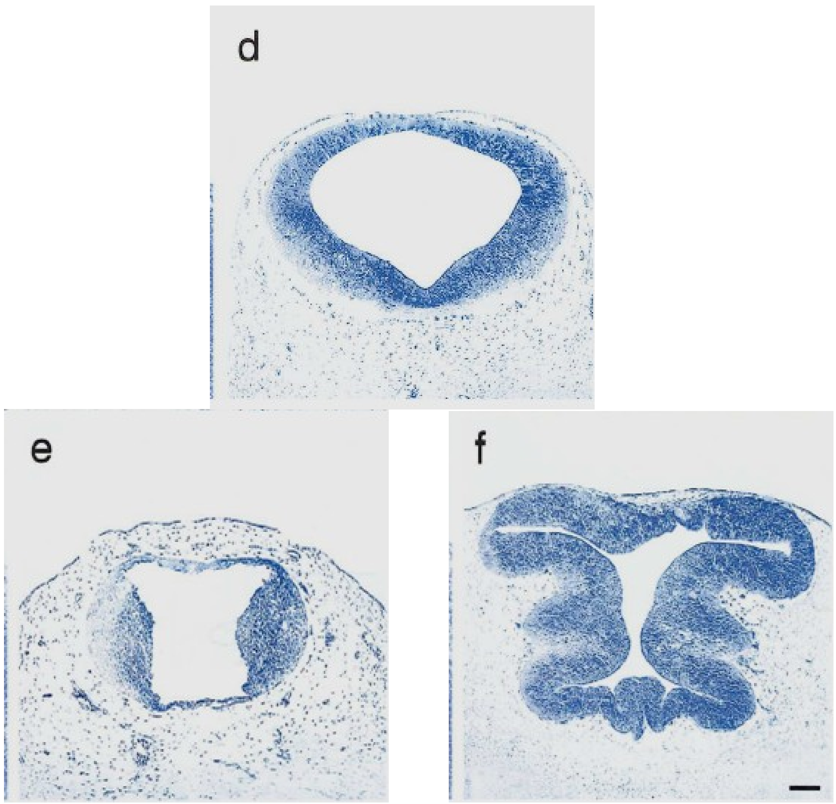
according to Beachy et al., Nature 2004

Wnt pathway		
Colon	Adenocarcinoma	Tumorigenesis by inactivation of APC, Axin; tumorigenesis by stabilization of β-catenin; epigenetic inactivation of SFRPs
Liver	Hepatoblastoma	Tumorigenesis (in mouse) by inactivation of APC and by stabilization of β-catenin
Blood	Multiple myeloma	Cell-growth inhibition by dominant negative TCF4; growth stimulation by Wnt ligand
Hair follicle	Pilomatricoma	Tumorigenesis (in mouse) by overexpression of β-catenin
Bone	Osteosarcoma	Dkk3 and LRP5 expression inhibits tumour cell growth *in vitro*
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short intefering RNA and a blocking antibody against Wnt2
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP

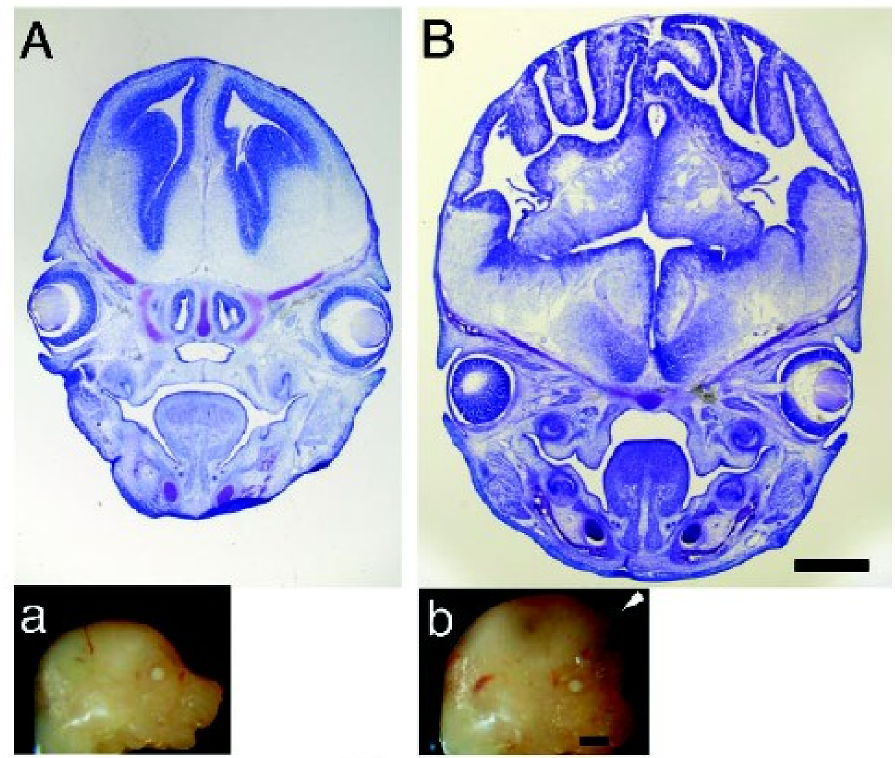
Aktivace β-cateninu ve vyvíjející se mozkové trubici:

midbrain (Brn4-promotor)

cortex (nestin enhancer)

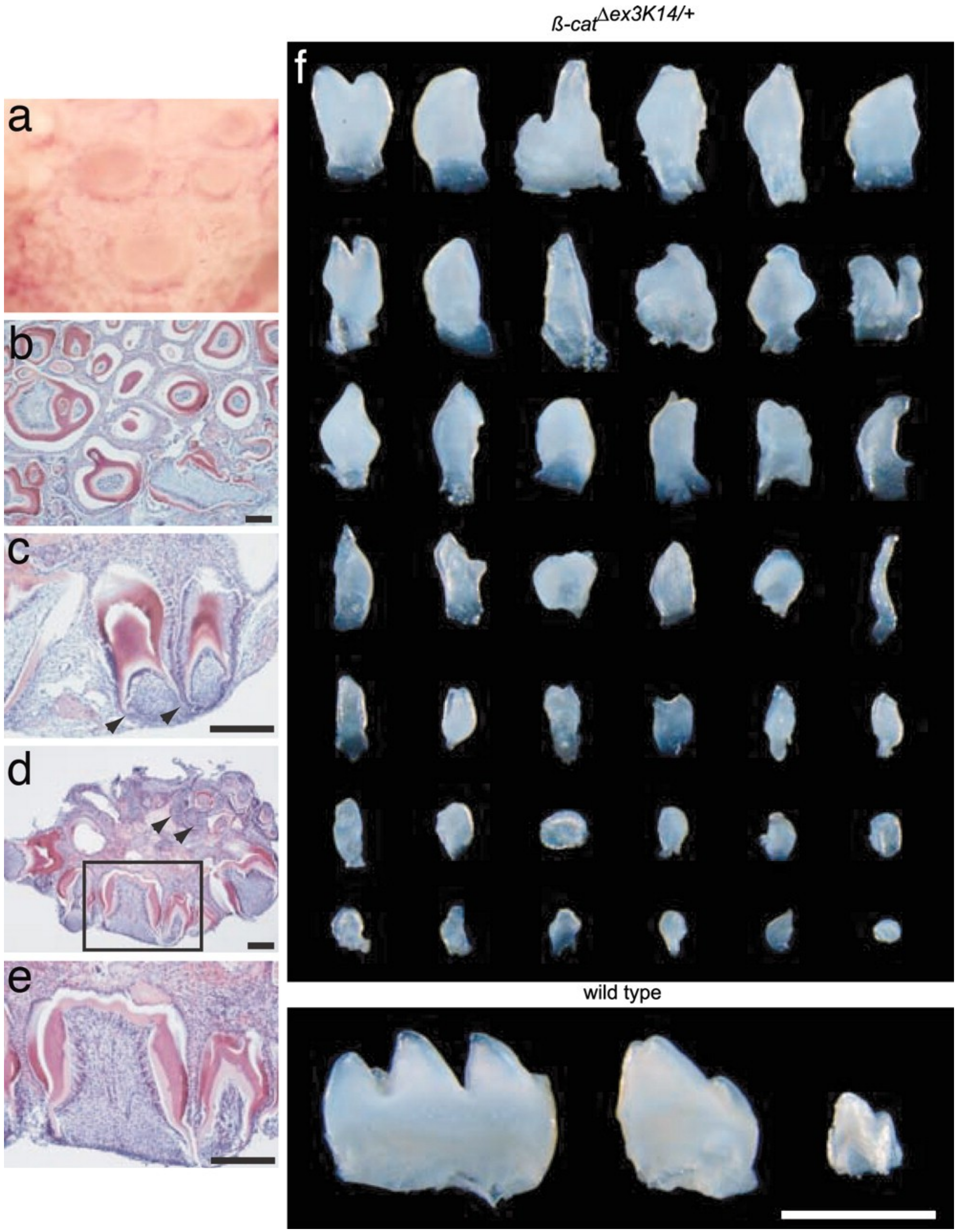


Zechner et al., 2003: Dev. Biol.;258:406-418.



Chenn & Walsh, 2002: Science;297:365-369.

Aktivace beta-cateninu v kmenových buňkách zubu:



Järvinenet al. (2006) Proc. Natl. Acad. Sci. USA 103, 18627-18632

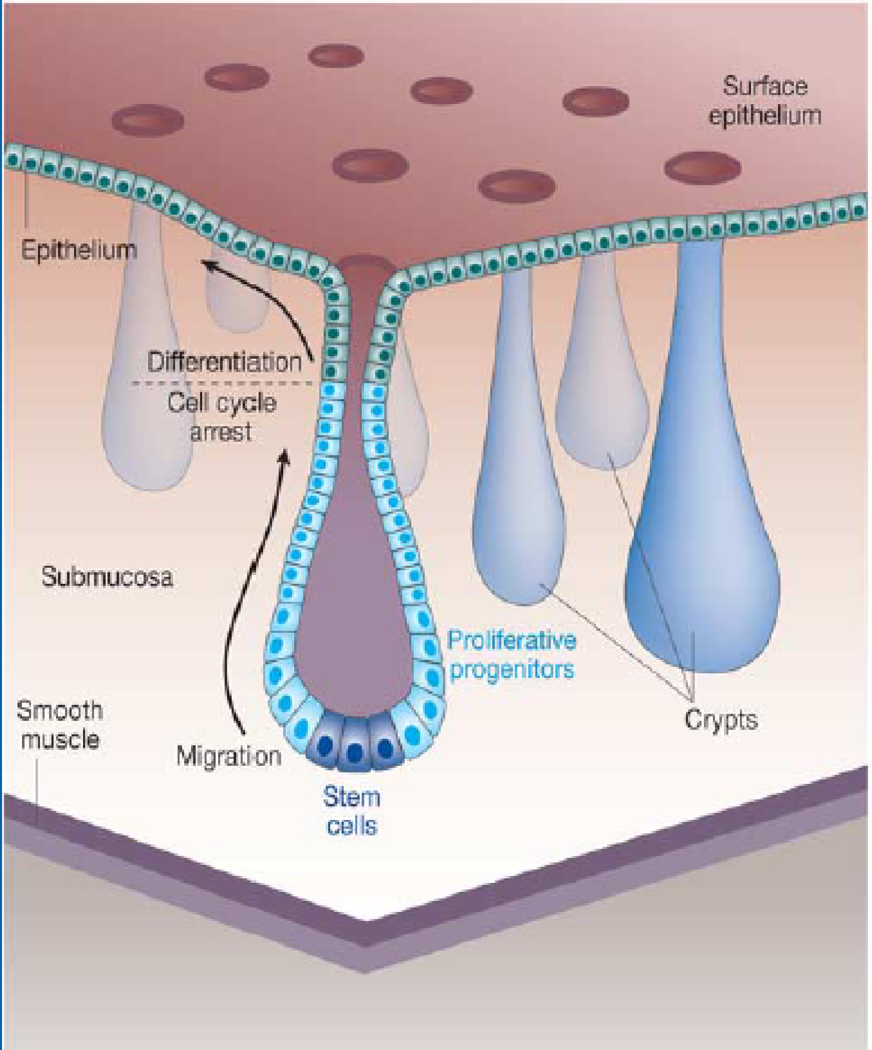


Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)

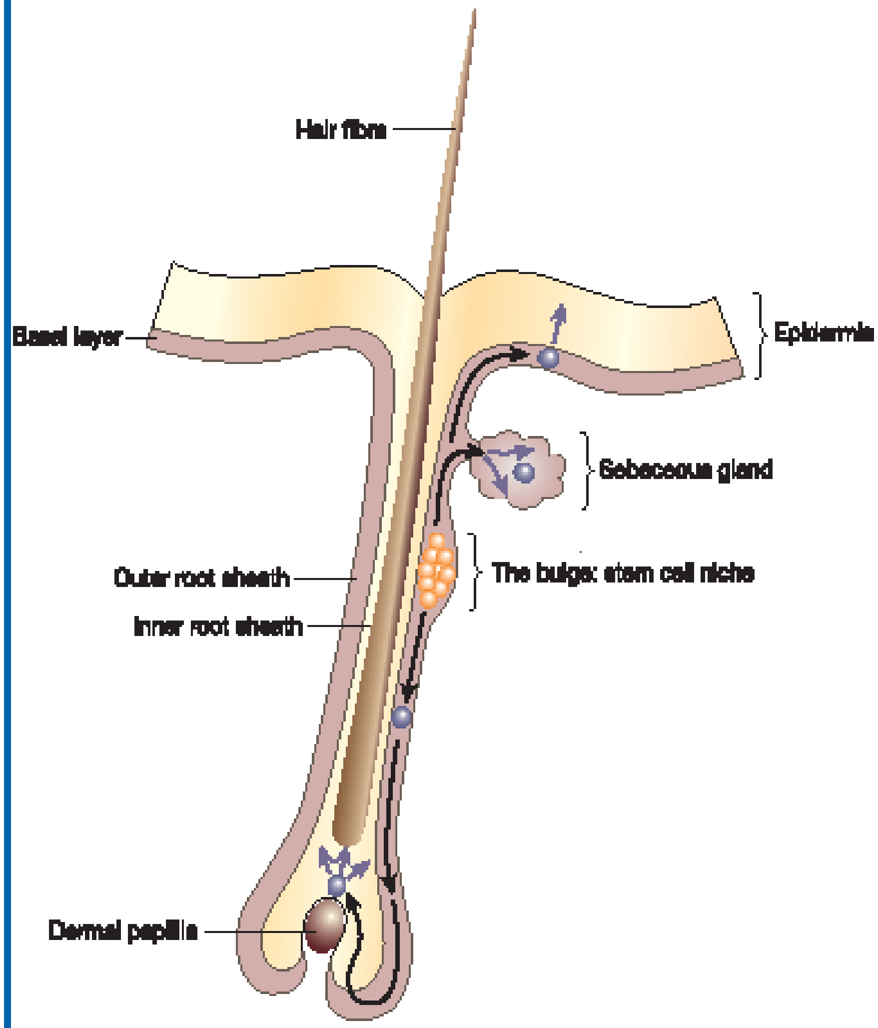
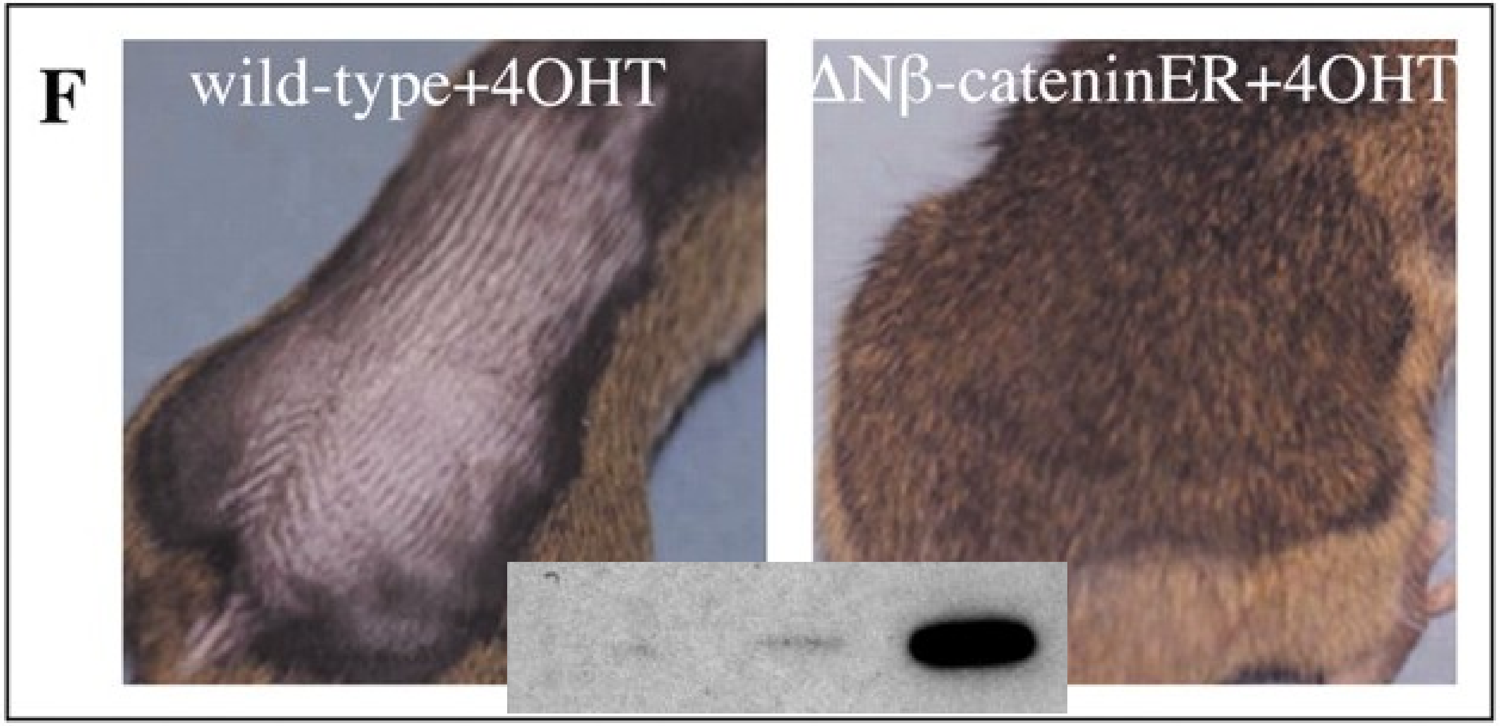


Figure 4 The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from ref. 90.)

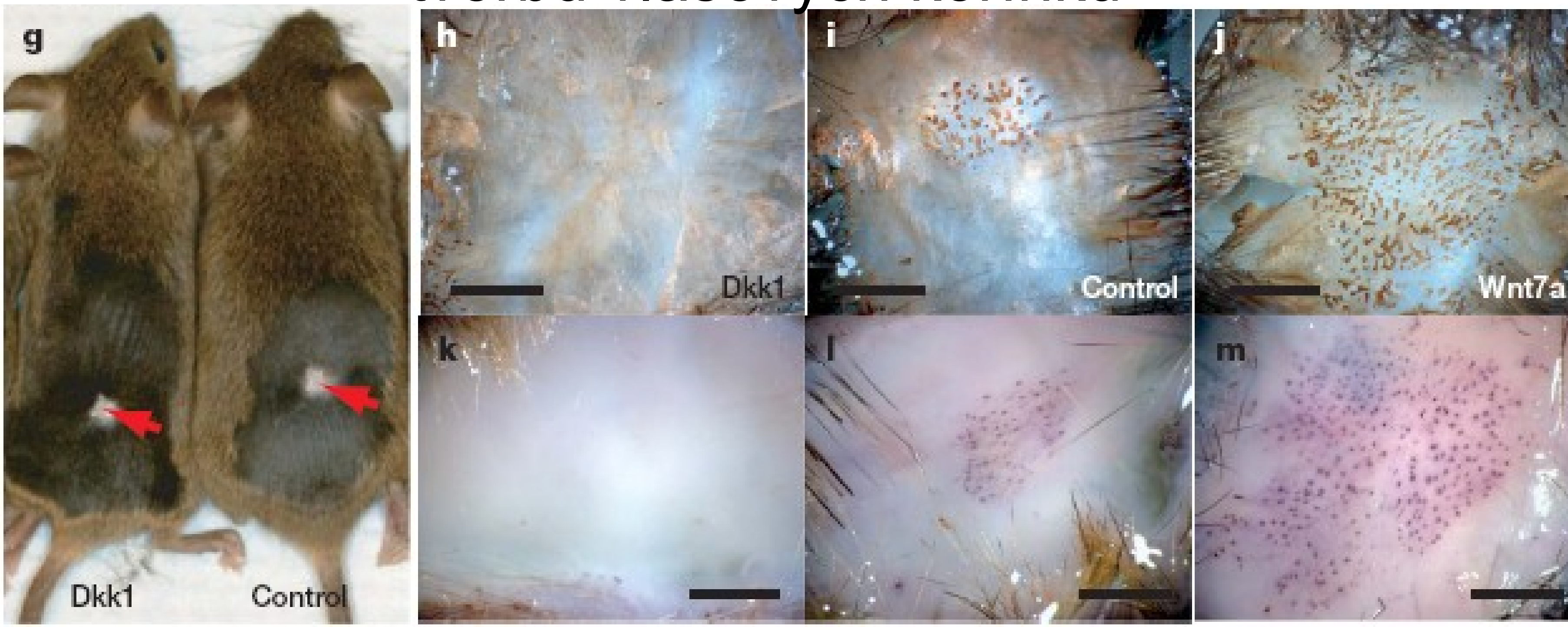
Reya & Clevers 2005, Nature

Důsledky aktivace β-cateninu v epidermis (po depilaci)



Lo Celso, C. L. et al. Development 2004;131:1787-1799

Aktivace kanonické Wnt dráhy indukuje de novo tvorbu vlasových kořínků



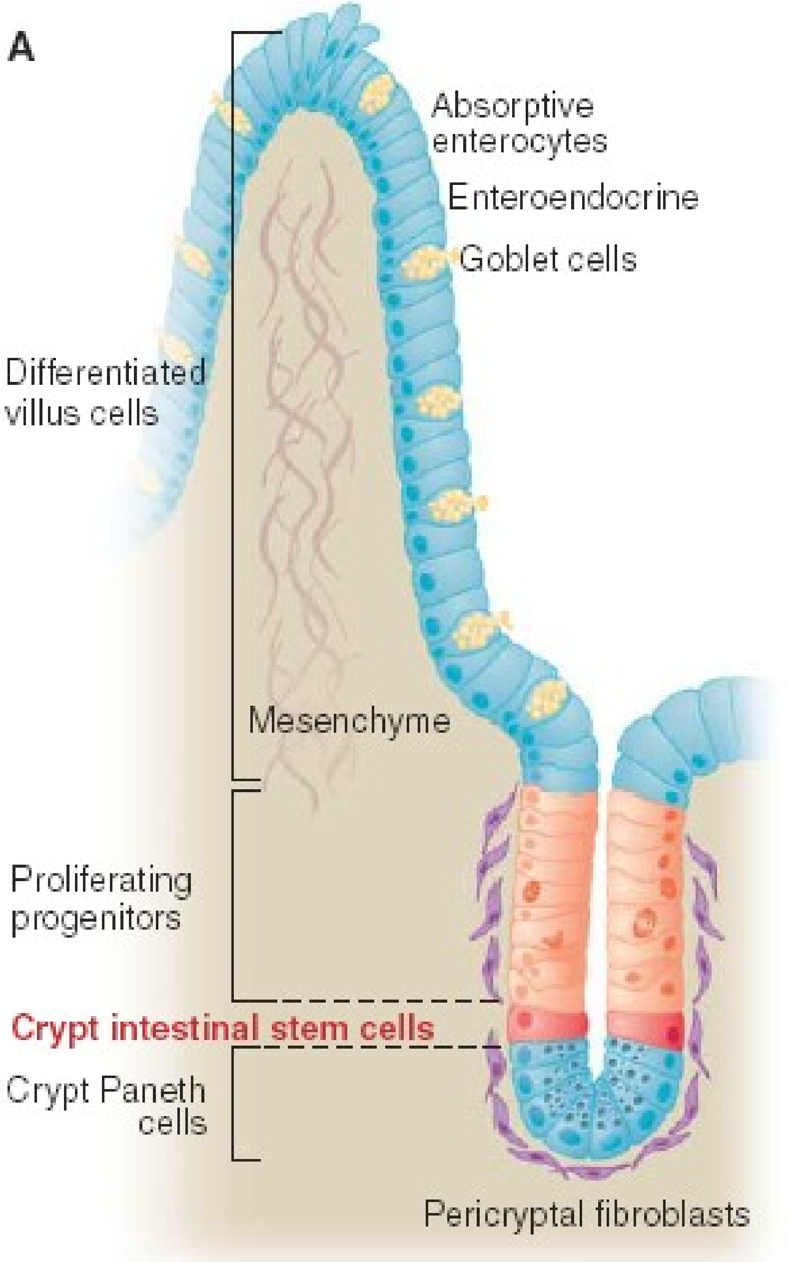
Wnt-dependent *de novo* hair follicle regeneration in adult mouse skin after wounding

Mayumi Ito[1], Zaixin Yang[1], Thomas Andl[1], Chunhua Cui[1], Noori Kim[1], Sarah E. Millar[1] & George Cotsarelis[1]

nature Vol 447|17 May 2007|doi:10.1038/nature05766

LETTERS

Wnt signalizace v normální a patologické fyziologii střevního epitelu



Moore & Lemischka, Science, 2006

Prostředí kmenových buněk (stem cell niche)

střevní epitel – latest developments aneb jak opravdu na to (Barker et al., Nature, October 2007)

A. Příprava transgenní myši č. 1 za účelem zjistit, kde je nový potenciální stem cell marker exprimován (in vivo expression profiling). Lgr5 je exprimován specificky v buňkách ve spodní části krypty.

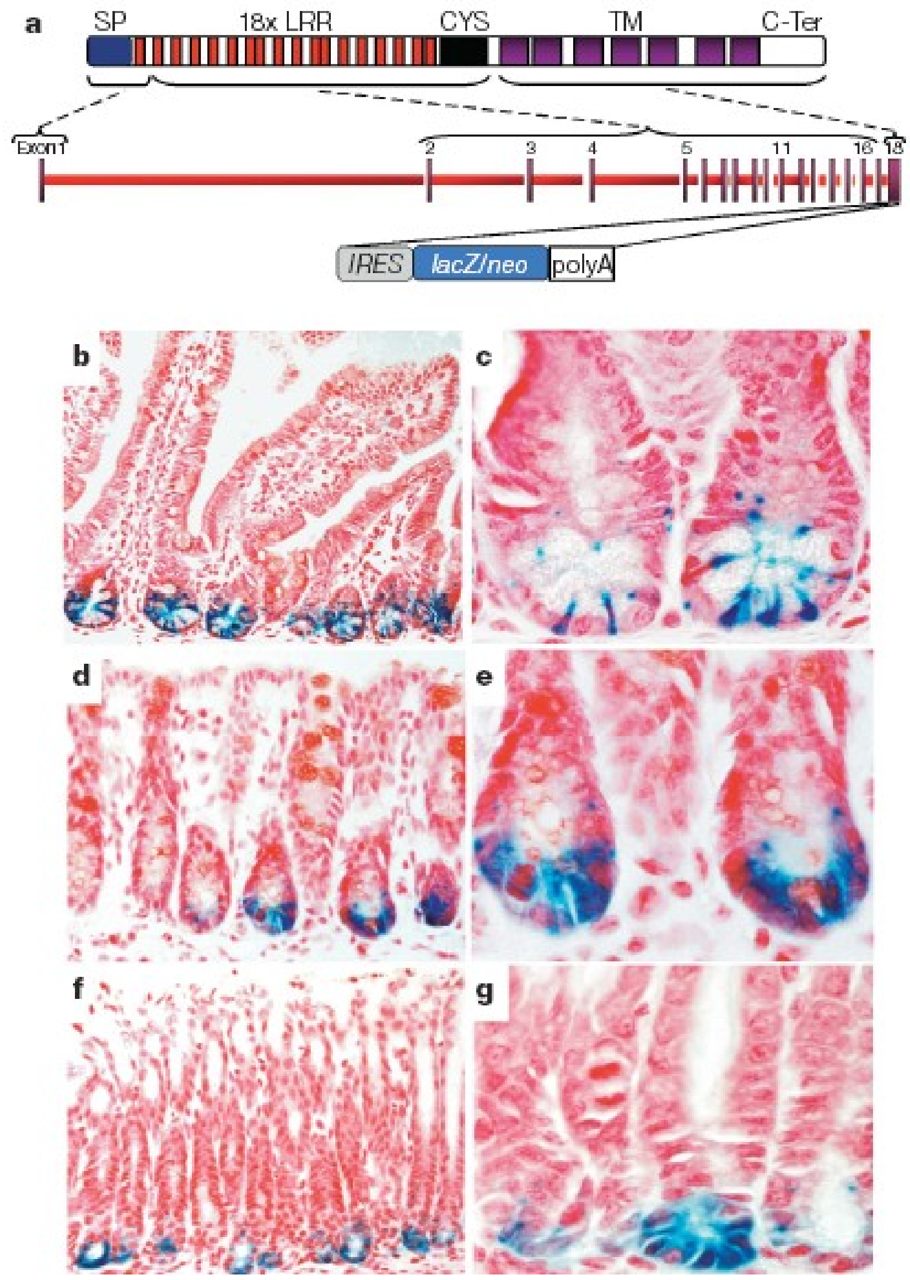


Figure 3 | Restricted expression of an *Lgr5-lacZ* reporter gene in adult mice. **a**, Generation of mice carrying *lacZ* integrated into the last exon of the *Lgr5* gene, removing all transmembrane (TM) regions of the encoded Lgr5 protein. Neo, neomycin resistance cassette; SP, signal peptide; LRR, leucine-rich repeat region; C-Ter is carboxy terminus. **b–h**, Expression of Lgr5-LacZ (blue) in selected adult mouse tissues. **b**, **c**, In the small intestine, expression is restricted to six to eight slender cells intermingled with the Paneth cells at the crypt base. **d**, **e**, In the colon, expression is confined to a few cells located at the crypt base. **f**, **g**, Expression in the stomach is limited to the base of the glands.

Prostředí kmenových buněk (stem cell niche)

střevní epitel – latest developments aneb jak opravdu na to (Barker et al., Nature, October 2007)

B. Příprava transgenní myši 2, 3 a 4 za účelem zjistit, co všechno vzniká z Lgr5-pozitivních buněk (Lgr5+ lineage tracing). Lgr5 pozitivní buňky dávají vzniknout všem částem buněčného epitelu.

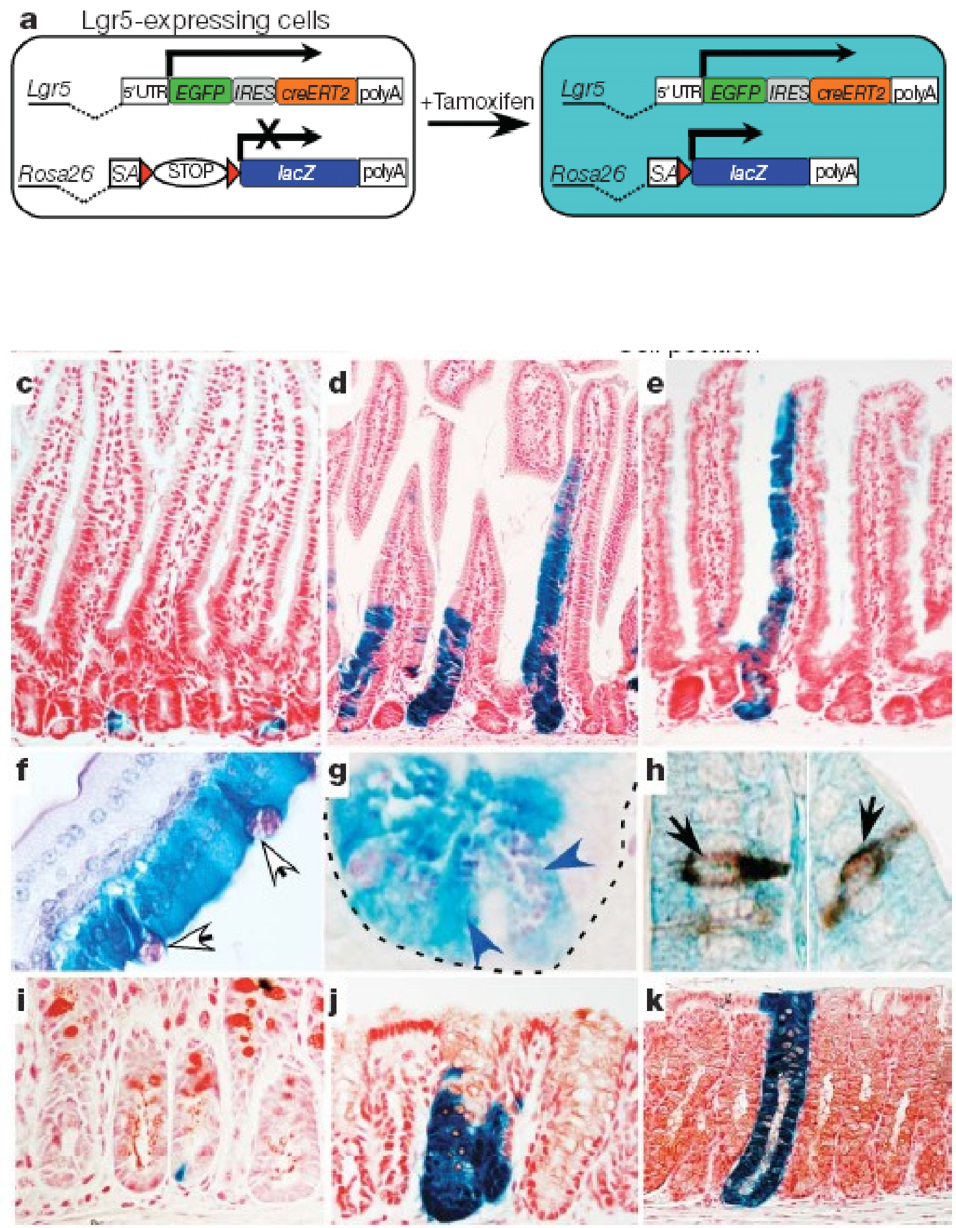
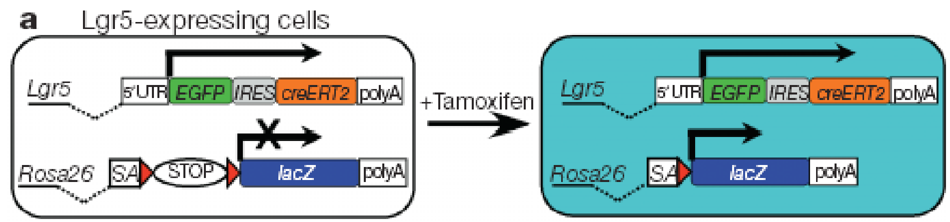


Figure 5 | Lineage tracing in the small intestine and colon. a, *Lgr5-EGFP-IRES-creERT2* knock-in mouse crossed with *Rosa26-lacZ* reporter mice 12 h after tamoxifen injection. **b,** Frequency at which the blue cells appeared at

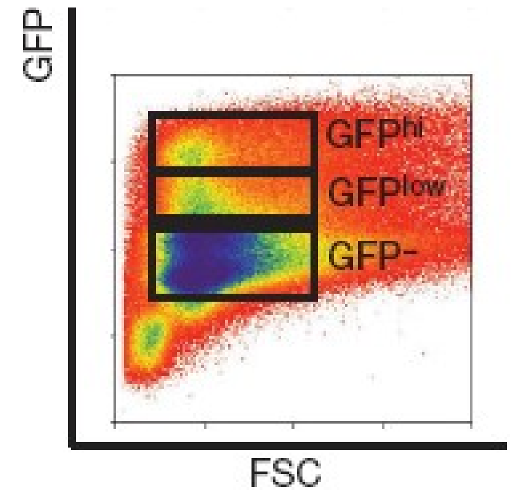
carrying activated Cre. **c–e,** Histological analysis of LacZ activity in small intestine 1 day after induction (**c**), 5 days after induction (**d**) and 60 days after induction (**e**). **f–h,** Double-labelling of LacZ-stained intestine using PAS demonstrates the presence of goblet cells (**f**, white arrows) and Paneth cells (**g**, blue arrows) in induced blue clones. Double-labelling with synaptophysin demonstrates the presence of enteroendocrine cells within the induced blue clones (**h**, black arrows). **i–k,** Histological analysis of LacZ activity in colon 1 day after induction (**i**), 5 days after induction (**j**) and 60 days after induction (**k**).

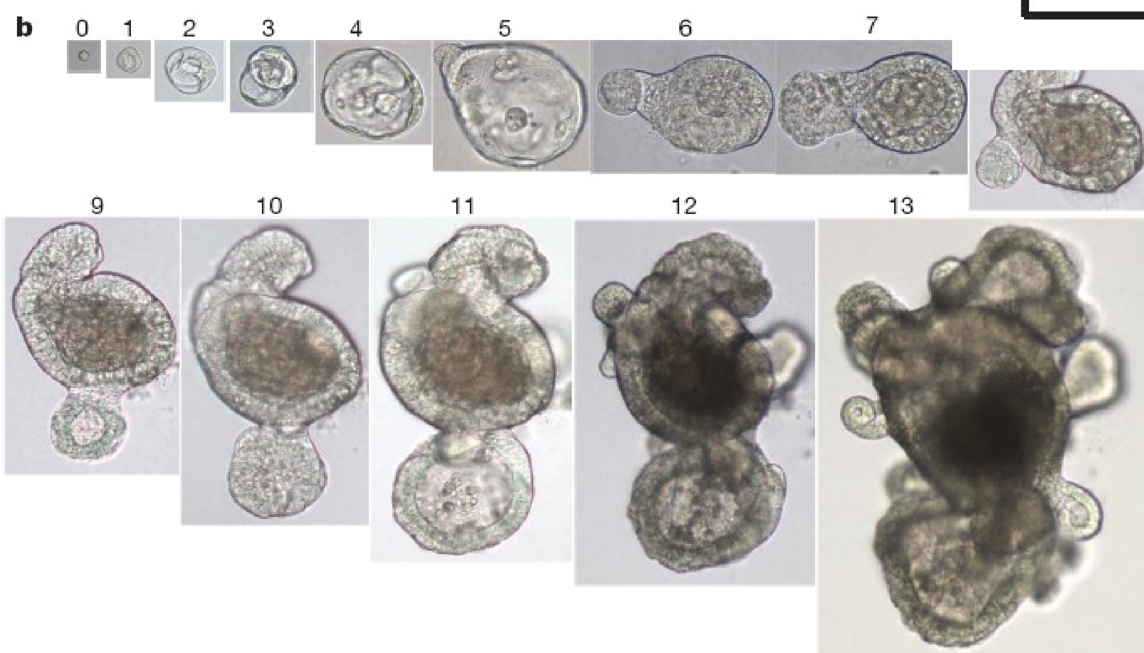
Prostředí kmenových buněk (stem cell niche)

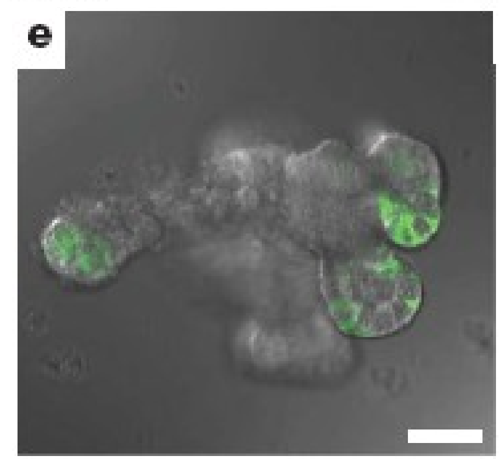


střevní epitel – latest developments aneb jak opravdu na to (Barker et al., Nature & Sato, Nature 2009)

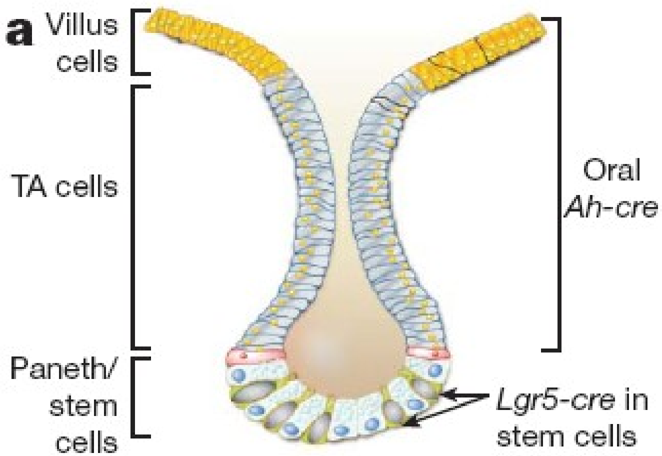
C. Lgr5 pozitivní buňky in vitro dávají vzniknout kompletní villus-crypt struktuře in vitro (Doposud se to s žádnými jinými buňkami nepodařilo)





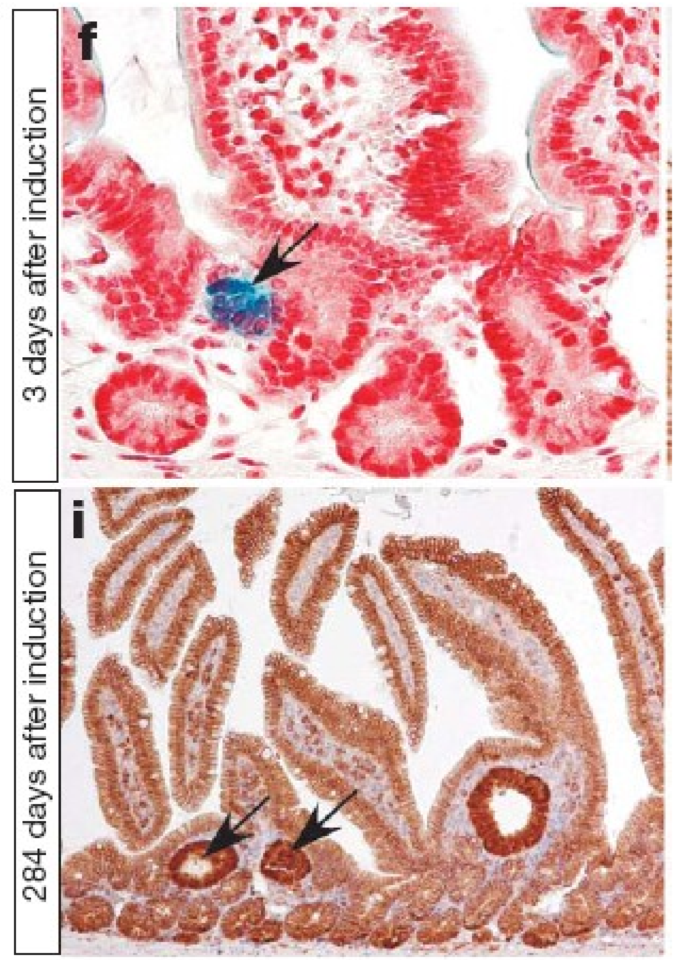


Experimentální důkaz:

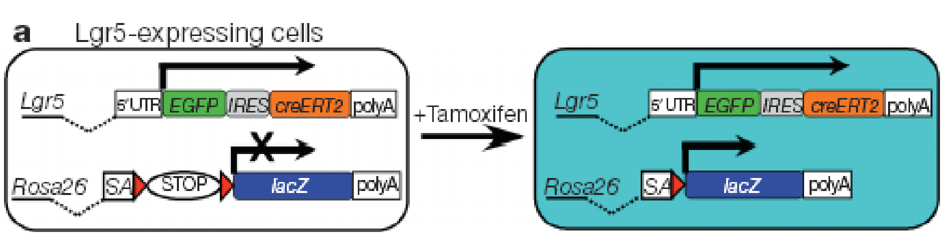


Zkřížení s APC flox/flox myšima + tamoxifen

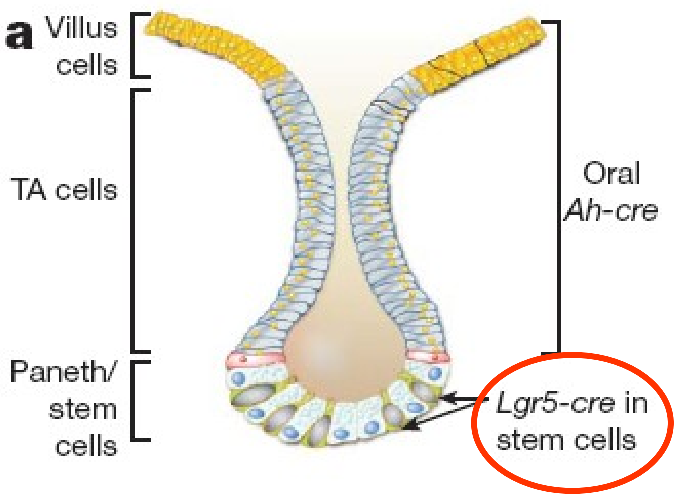
Vznik malých adenomů, které neprogredují



NATURE|Vol 457|29 January 2009

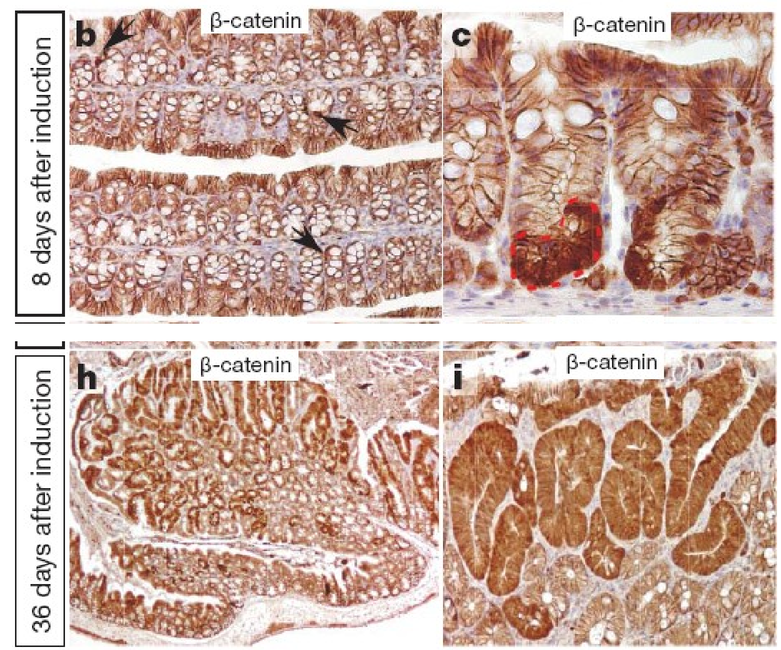


Nekontrolovaná aktivace kmenových buněk má fatální následky



Zkříažení s APC flox/flox myšima + tamoxifen

Rychlý vznik adenomů a nádorů



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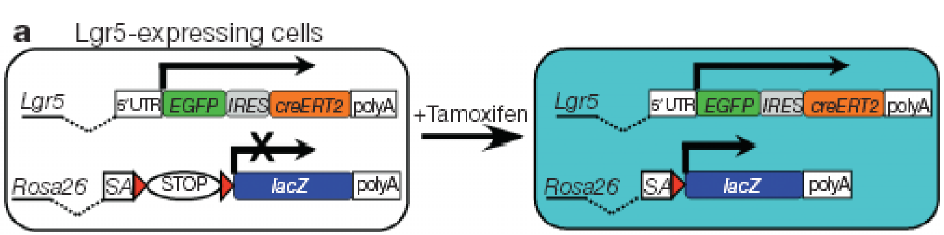
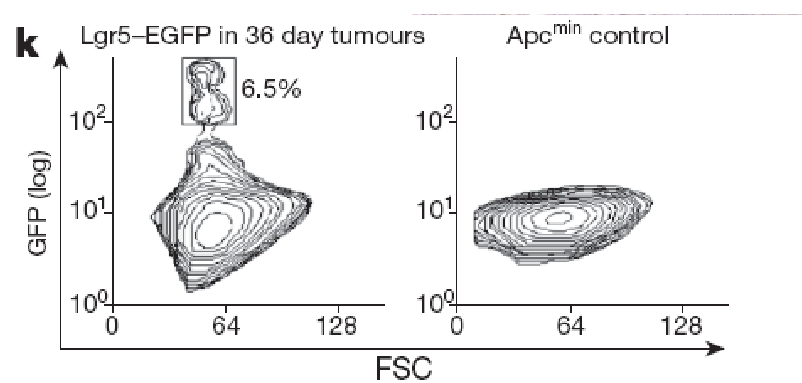
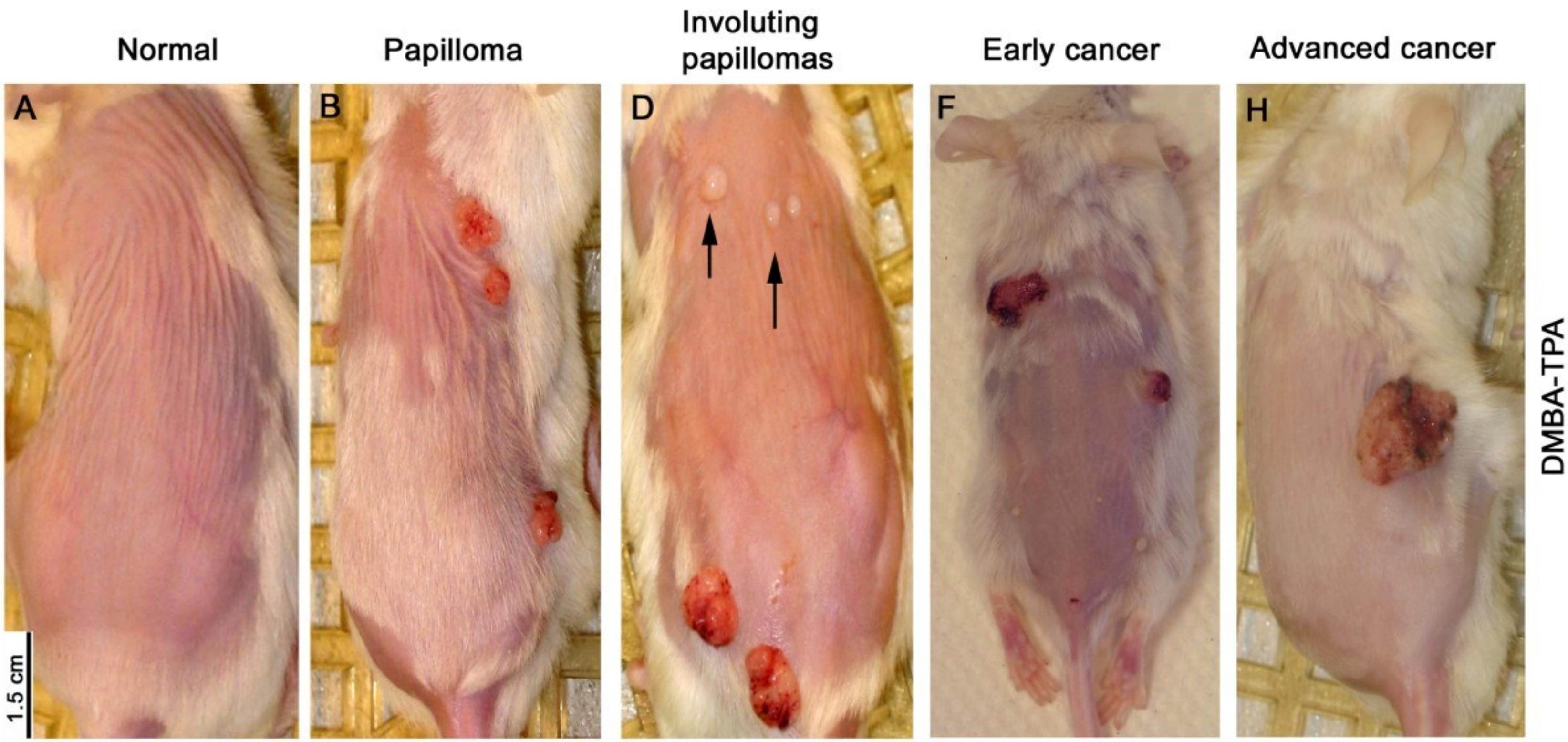




DMBA/TPA-induced skin carcinogenesis

- benign papilomas, which in some cases progress into squamous cell carcinoma (SCC)



DMBA/TPA-induced skin carcinogenesis

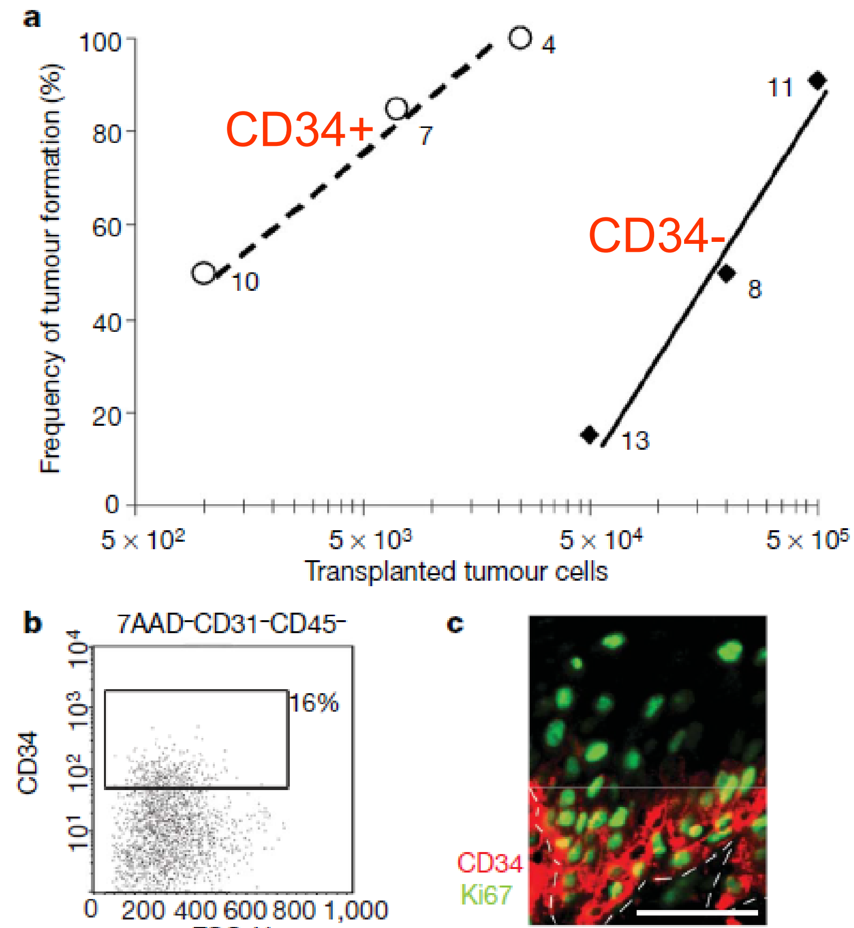
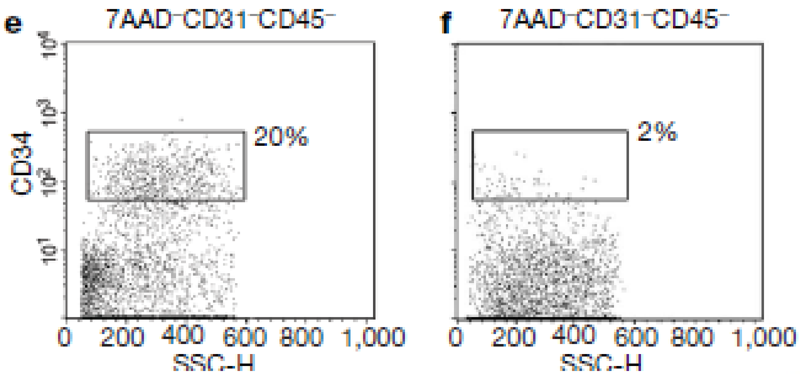
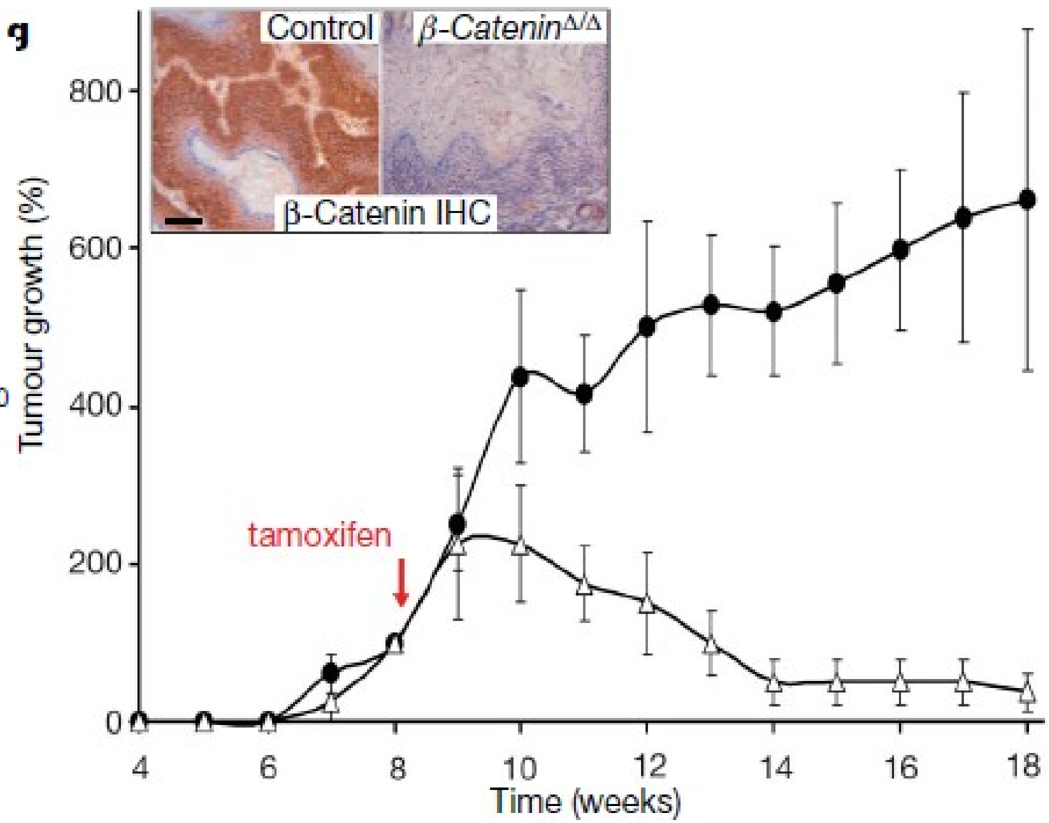


Figure 2 | Cancer stem cells efficiently initiate secondary tumours that recapitulate the organization of the primary tumour. a, Diagram summarizing the frequency of tumour formation in orthotopic tumour transplants using unsorted cells (filled diamonds) or $CD34^+$ cells (open circles) in varying amounts. The *n* value for each point is shown. **b,** Abundance of CSCs ($CD34^+7AAD^-CD31^-CD45^-$) in secondary tumours derived from orthotopic transplantations of $CD34^+$ cells.

Malanchi et al. 2008, Nature



characterized by abundant keratinization. **e**, **f**, Abundance of CD34$^+$ cells in control tumours (**e**) and β-catenin-deficient tumours (**f**) two weeks after induction of deletion. **g**, Tumour regression after tamoxifen-induced β-catenin deletion in established skin tumours (n = 6; control (filled circles), *K14-creER*^{T2}*:β-catenin*$^{+/\Delta}$; mutant (open triangles), *K14-creER*^{T2}*:β-catenin*$^{\Delta/\Delta}$). Tumour numbers at 8 weeks were set to 100%. Error bars show

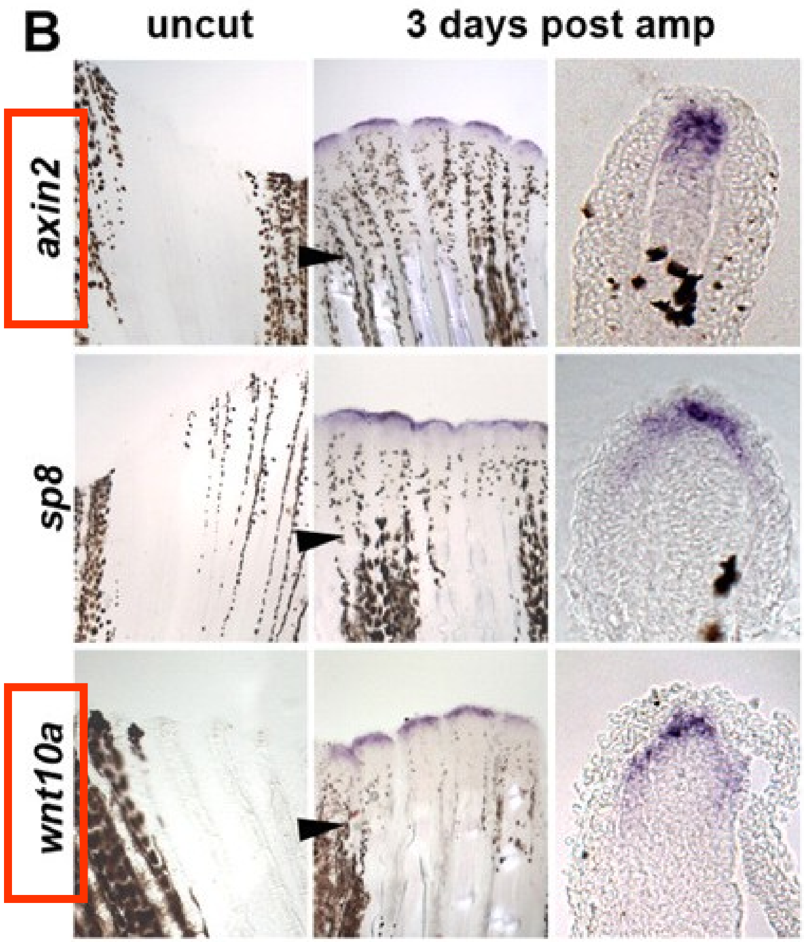


Malanchi et al. 2008, Nature

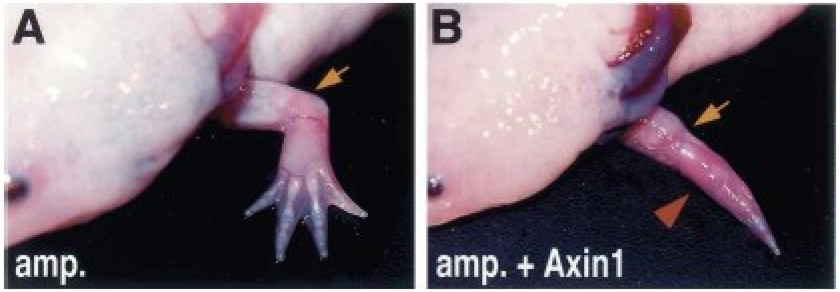
Wnt/β-cateninová dráha v regeneraci

II. regenerace

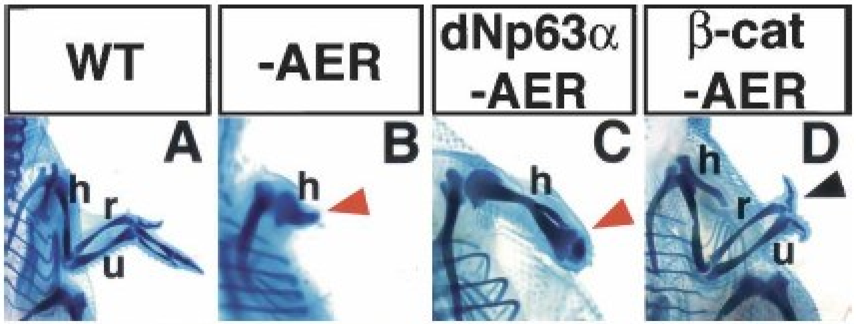
zebrafish



axolotl



chick



Wnt/β-cateninová dráha v regulaci stárnutí

Augmented Wnt Signaling in a Mammalian Model of Accelerated Aging

Hongjun Liu,[1] Maria M Fergusson,[1*] Rogerio M. Castilho,[2*] Jie Liu,[1] Liu Cao,[1] Jichun Chen,[3] Daniela Malide,[4] Ilsa I. Rovira,[1] Daniel Schimel,[5] Calvin J. Kuo,[6] J. Silvio Gutkind,[2] Paul M. Hwang,[1] Toren Finkel[1]†

SCIENCE VOL 317 10 AUGUST 2007 803

Increased Wnt Signaling During Aging Alters Muscle Stem Cell Fate and Increases Fibrosis

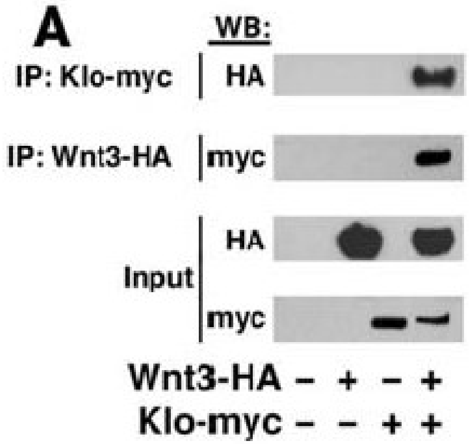
Andrew S. Brack,[1] Michael J. Conboy,[1] Sudeep Roy,[1] Mark Lee,[2] Calvin J. Kuo,[2] Charles Keller,[3] Thomas A. Rando[1,4]*

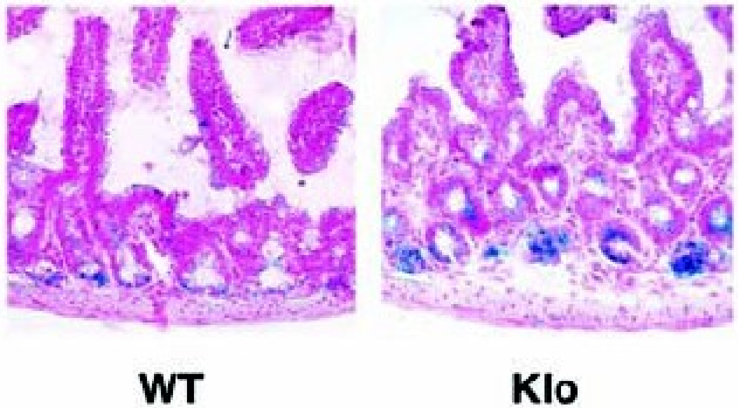
SCIENCE VOL 317 10 AUGUST 2007 807

Klotho myš

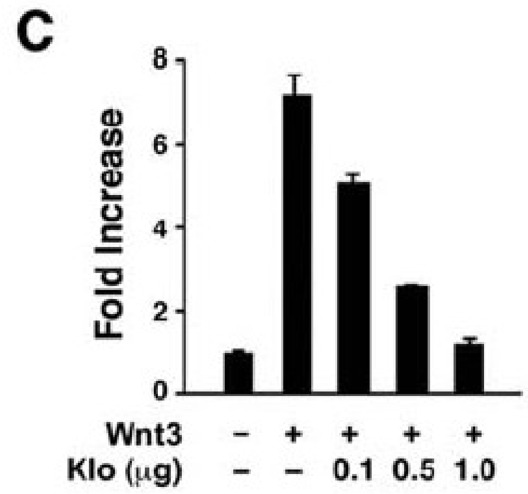
-mutantní kmen myši s fenotypem akcelerovaného stárnutí: např. kratší život, arterioskleróza, snížená plodnost nebo kožní atrofie

- protein Klotho je transmembránový protein s velkou extracelulární doménou, ta může být odštěpena a volně cirkulovat v krvi



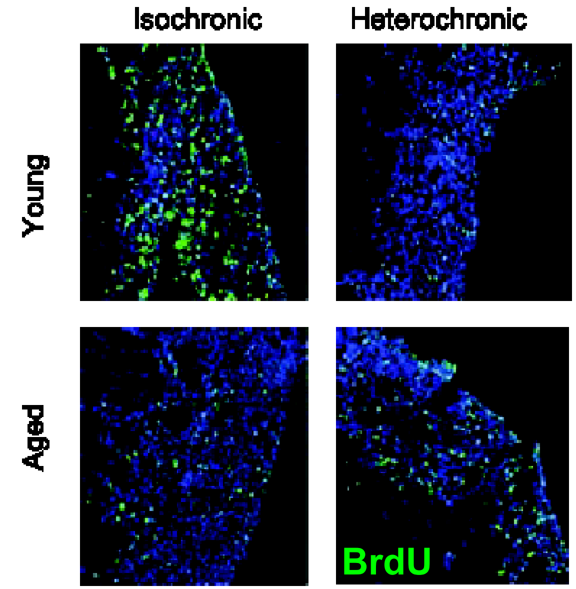


aktivita Wnt/β-cateninové dráhy
ve střevním epitelu



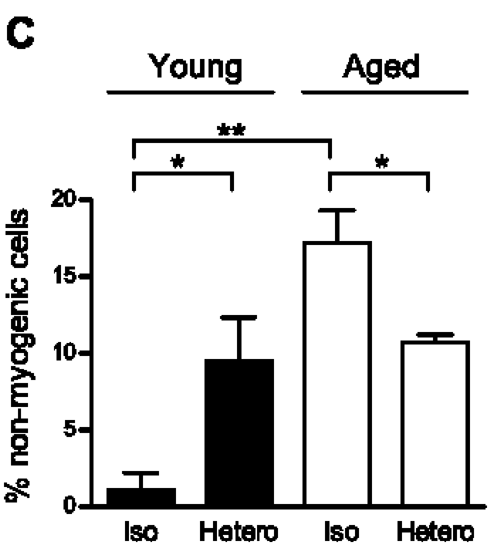
Model 1 – svalová fibróza

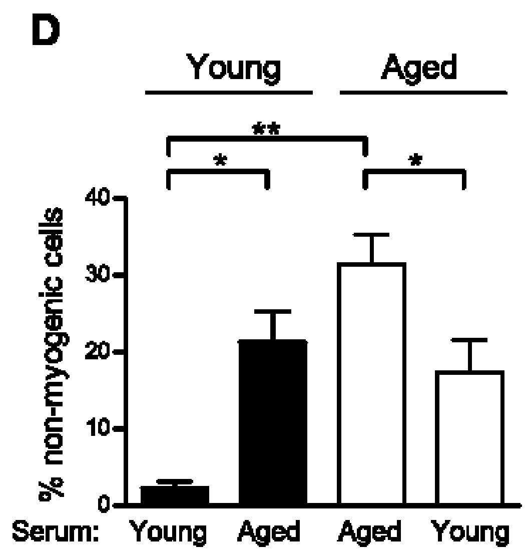
- s prodlužujícím se věkem stále častěji při regeneraci svalu vznikají místo svalových buněk buňky fibrózní tkáně – tak přispívají k nižší výkonnosti svalu, která souvisí se stárnutí

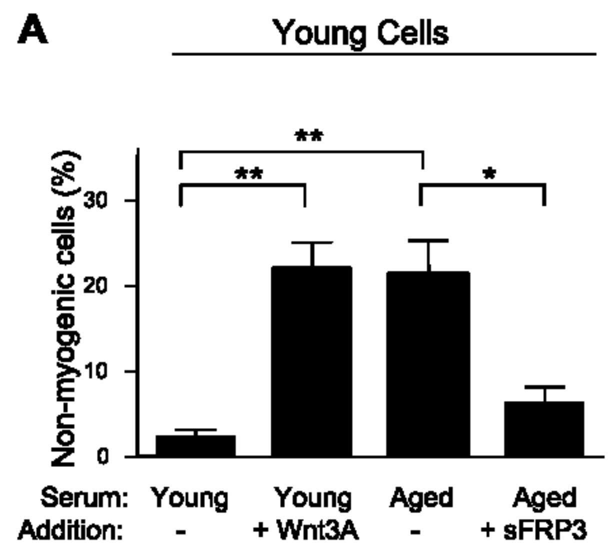


Model 2 – parabiotické párování

Fyzické propojení dvou krevních systémů (a tím i dvou vnitřních prostředí) u myši



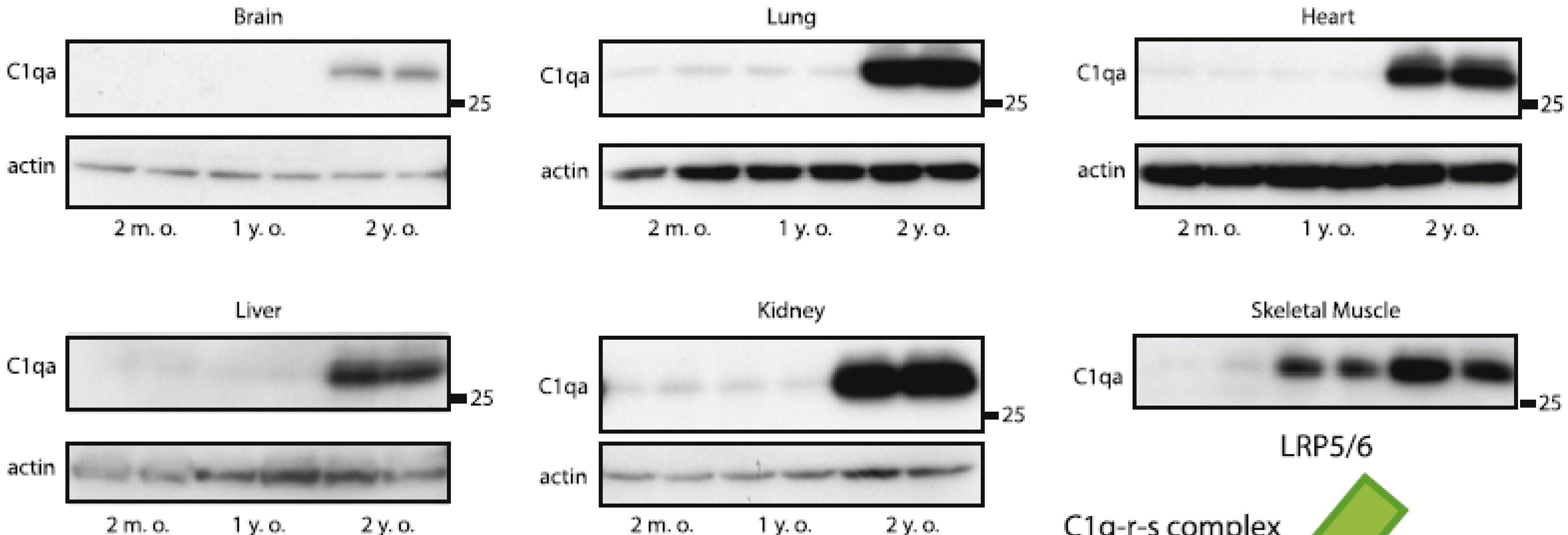


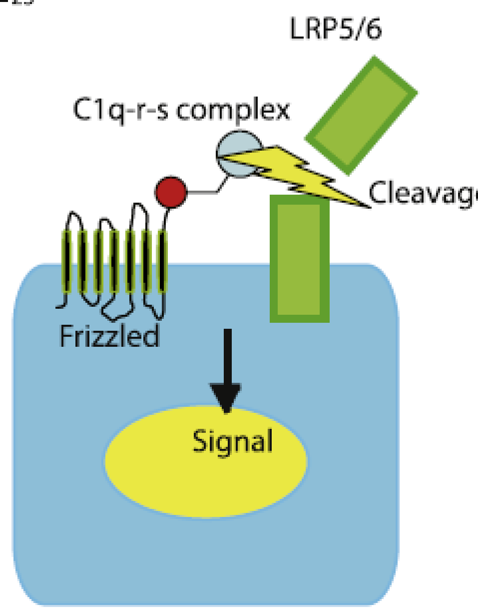


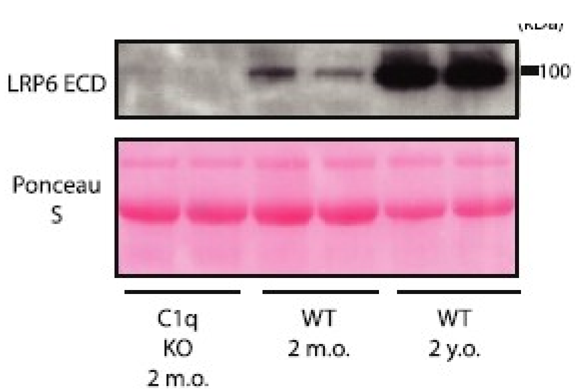
Cell

Complement C1q Activates Canonical Wnt Signaling and Promotes Aging-Related Phenotypes

Atsuhiko T. Naito,[1,3] Tomokazu Sumida,[4] Seitaro Nomura,[4] Mei-Lan Liu,[4] Tomoaki Higo,[1] Akito Nakagawa,[1] Katsuki Okada,[1] Taku Sakai,[1] Akihito Hashimoto,[1] Yurina Hara,[1] Ippei Shimizu,[4] Weidong Zhu,[4] Haruhiro Toko,[4] Akemi Katada,[4] Hiroshi Akazawa,[1,3] Toru Oka,[1,3] Jong-Kook Lee,[1,3] Tohru Minamino,[4] Toshio Nagai,[4] Kenneth Walsh,[5] Akira Kikuchi,[2] Misako Matsumoto,[6] Marina Botto,[7] Ichiro Shiojima,[1,3] and Issei Komuro[1,3,4,*]

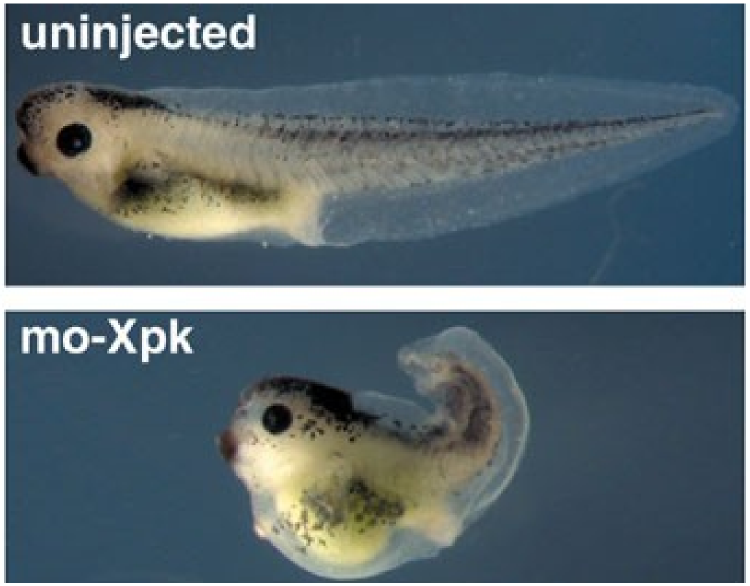






Nekanonická Wnt dráha

- e.g. Wnt5a

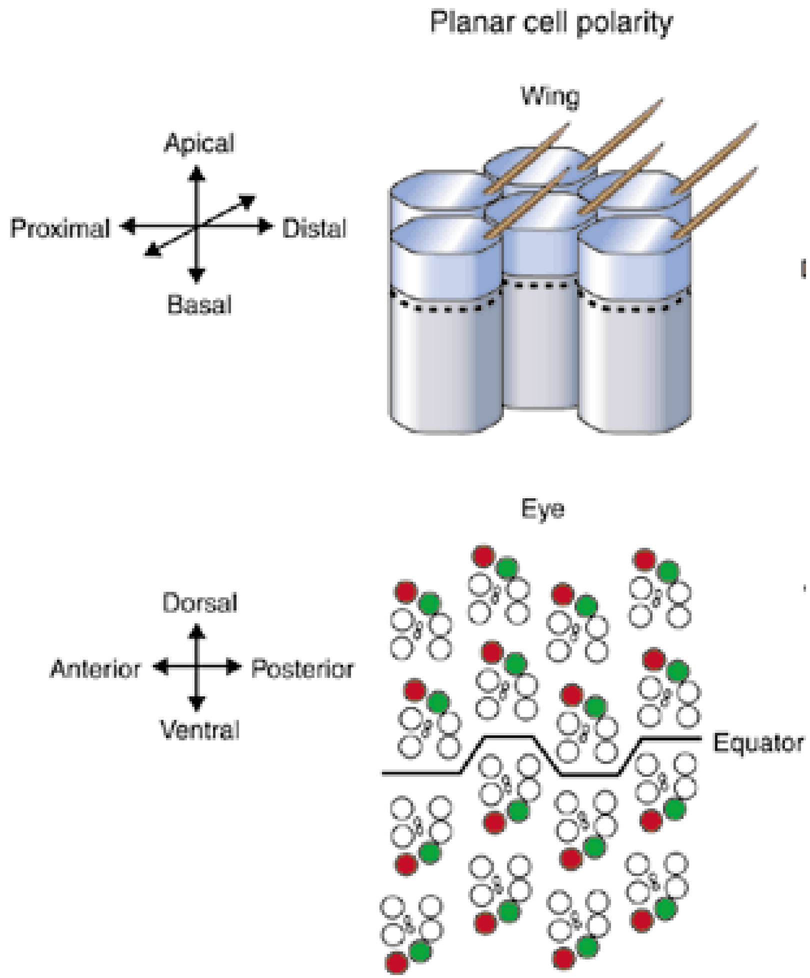


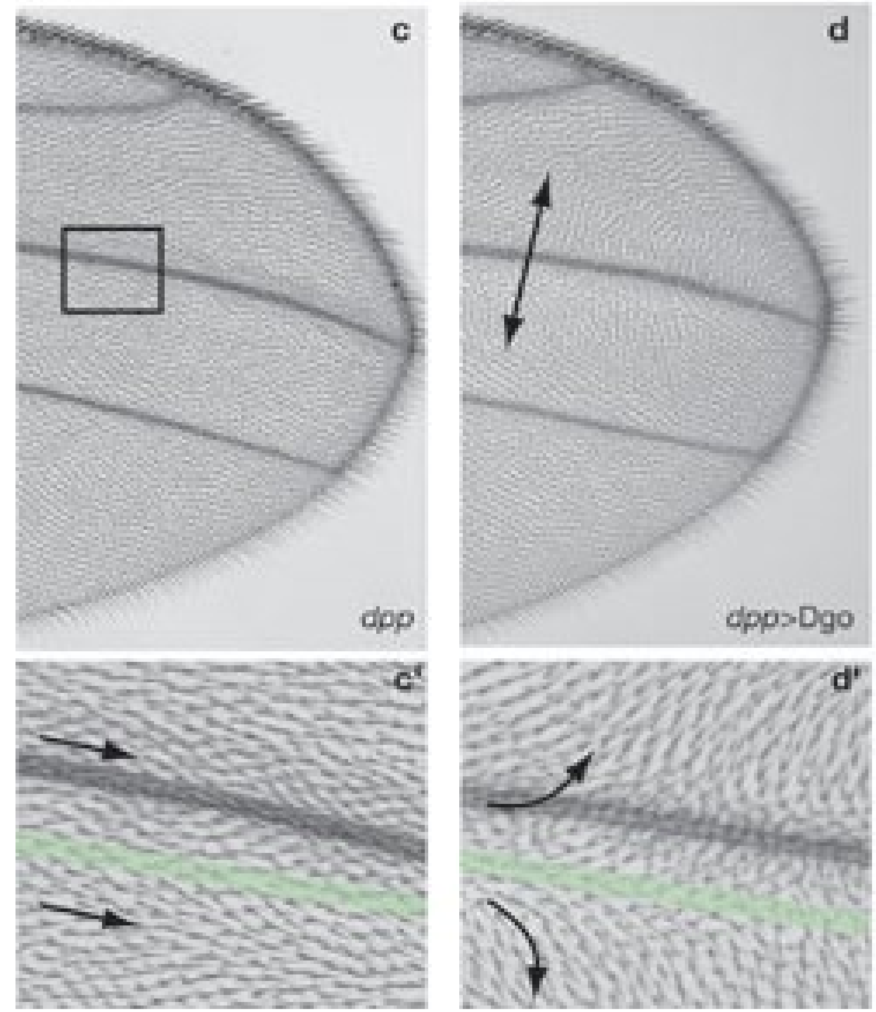
- do not induce axis duplication in Xenopus

- do not induce transformation of mammary cell line C57mg

- do not signal via nuclear translocation of β-catenin

Drosophila – PCP (planar cell polarity)

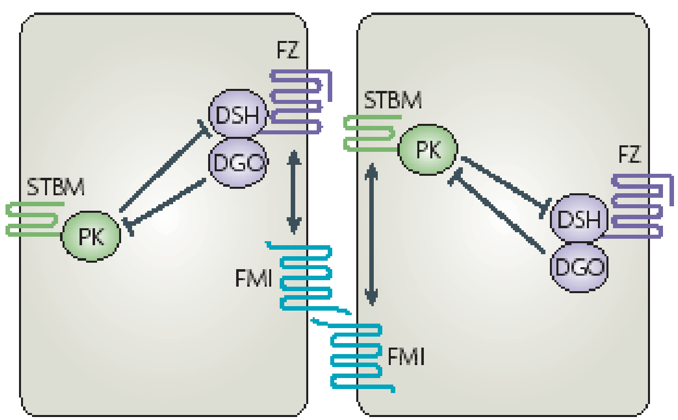




Molekulární mechanismus ustavení PCP

Box 1 | **Molecular interactions between the Fz/PCP core factors**

The molecular logic of the formation and separation of the Frizzled–Dishevelled–Diego (FZ–DSH–DGO) and Prickle–Strabismus (PK–STBM) complexes has started to be unravelled. In FIG. 2 are reported examples of the localization of each complex in various tissues. The figure is an apical view of two cells that have attained asymmetric localization of the two complexes. Several lines of



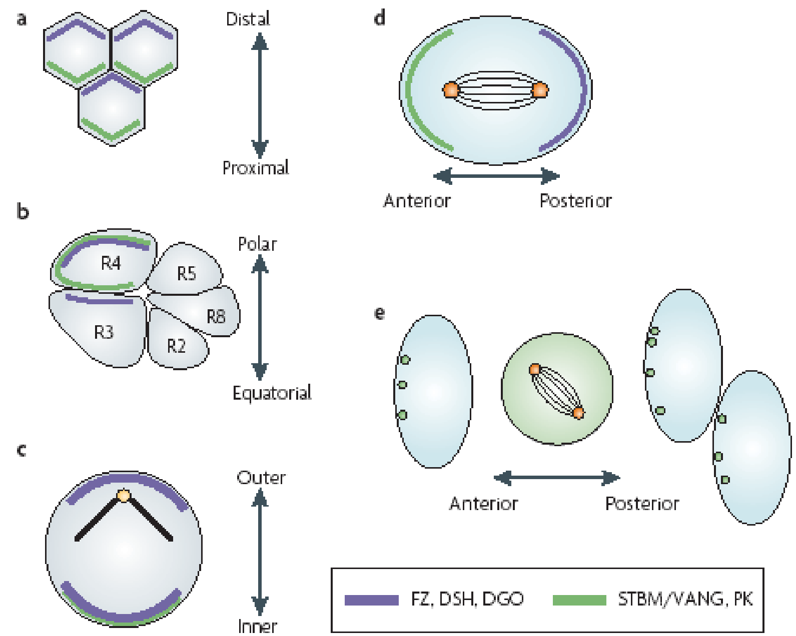
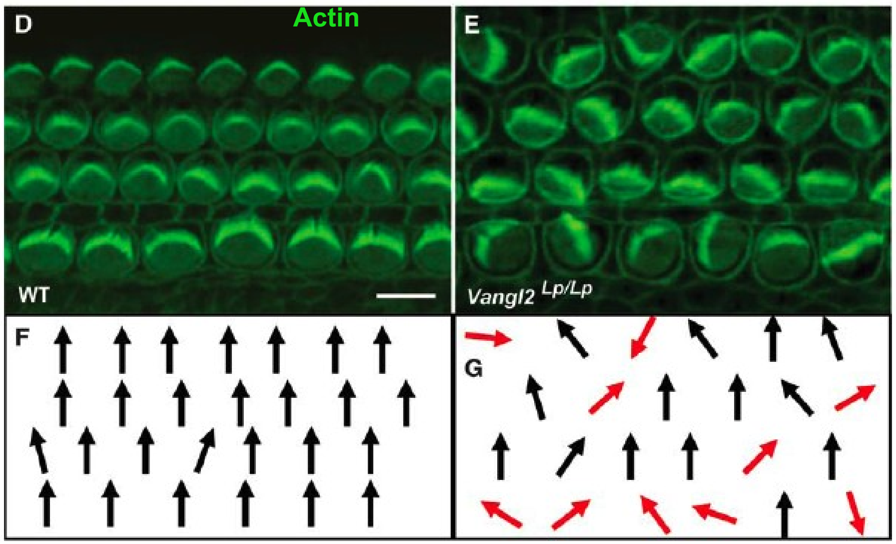


Figure 2 | **Subcellular distribution of core Fz/PCP factors in *Drosophila melanogaster* and vertebrates. a–c** | Examples of cells with epithelial character (marked by grey shading). *Drosophila melanogaster* wing cells and eye R3 and R4 cells and mouse sensory hair cells in the cochlea (inner ear) are shown in **a**, **b** and **c**, respectively. **d**,**e** | Examples of dividing cells. The spindle orientation in the *D. melanogaster* sensory organ precursor (SOP) cells depends on the asymmetric distribution of the Frizzled (Fz)/planar cell polarity (PCP) factors (as shown in **d**), as does the orientation of neuroectodermal cells in zebrafish (as shown in **e**; note that during mitosis the asymmetric distribution of PK is lost and then re-established). Depending on the tissue, only a subset of the respective proteins has been analysed (the *D. melanogaster* wing is the only tissue in which all proteins were analysed; all but DSH have been analysed in the eye). These illustrations represent the localizations patterns of PCP proteins at the proposed time of signalling. In the wing, asymmetry of Flamingo (FMI) has been reported earlier, but the relevance of this is unknown[52]. Note that in the mouse inner ear (as shown in **c**) vang-like 2 (VANGL2) and FZ3/FZ6 localize to the same side of the cells; it is not known whether other Fz family members localize with the DSH homologues DVL1 and DVL2 to the opposite side. During zebrafish gastrulation (as shown in **e**) Prickle (Pk), which is represented by green circles, is cytoplasmic during cell division but regains polarity after separation of the daughter cell. Only PK has been analysed in this context, but its localization depends on the presence of Strabismus (STBM).

Seifert and Mlodzik, Nature Reviews in Genetics, 2007

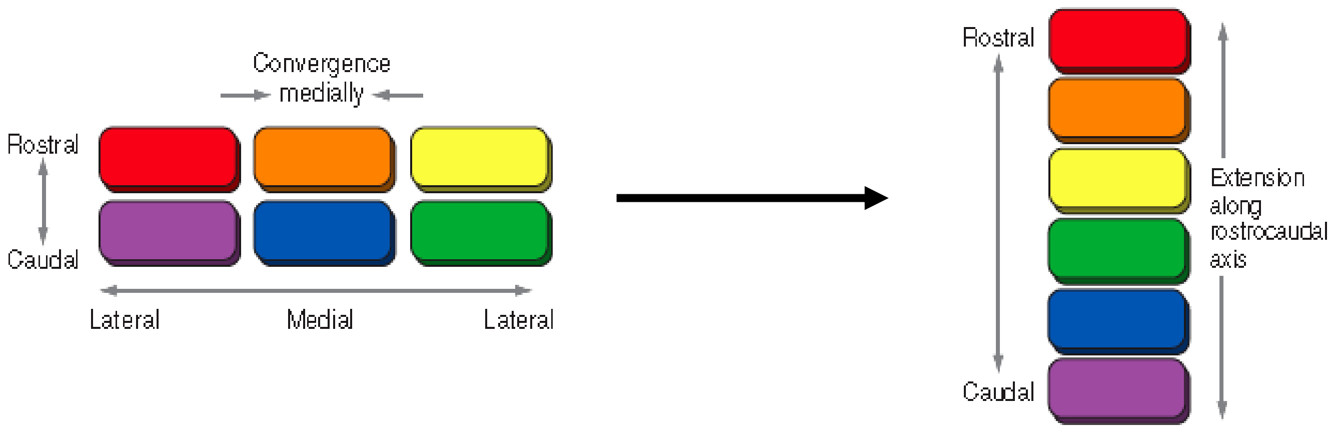
Non-canonical/PCP (Planar cell polarity) pathway: phenotypes in mouse

Stereocilia orientation in inner ear hair cells



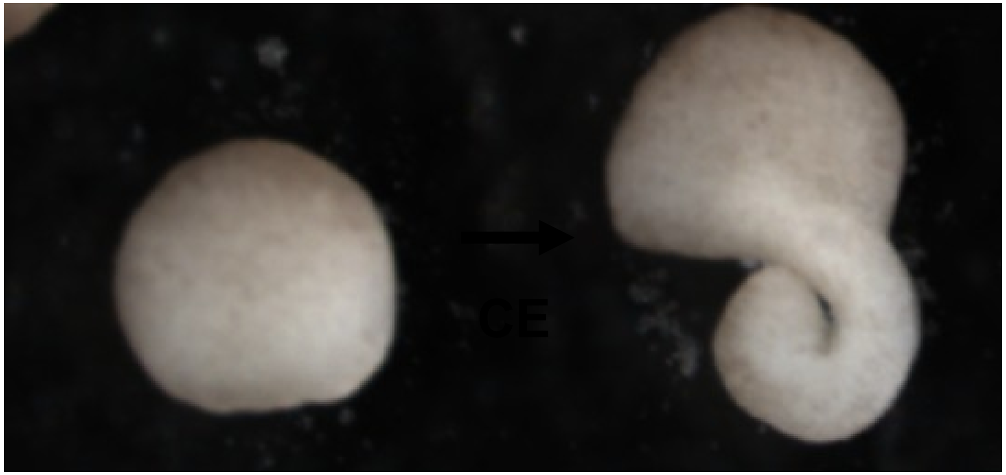
Qian et al., 2007, Dev. Biol.

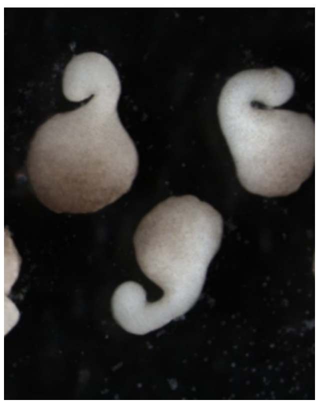
Non-canonical/PCP (Planar cell polarity) in mouse (and human) convergent extension

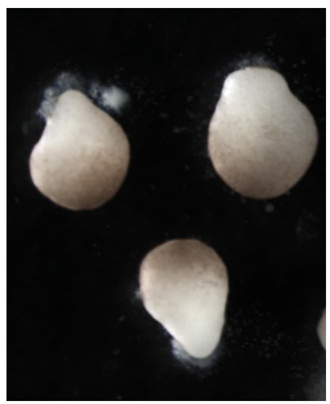


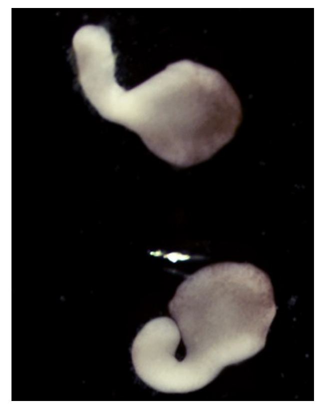
Konvergentní extenze – migrace buněk směrem ke středu těla – vede k prodlužování tělní osy

Možnosti studia konvergentní extenze - Kellerovy explantáty (Xenopus)









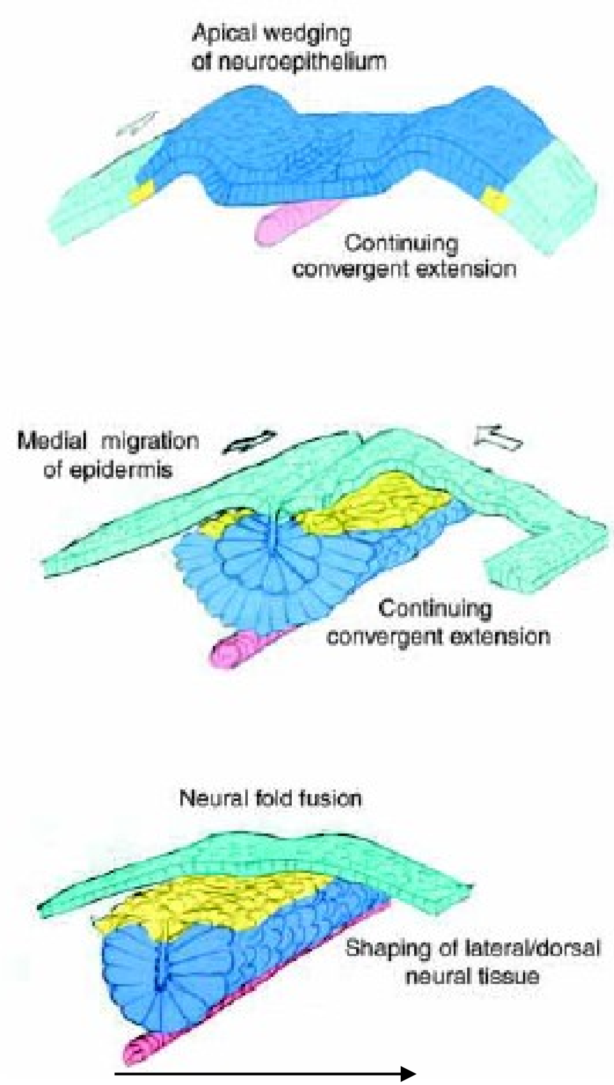
control

XLRP5 MO

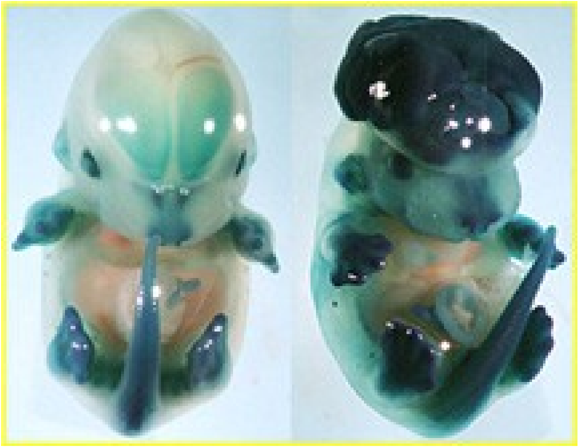
XLRP5 MO

+ mLrp5

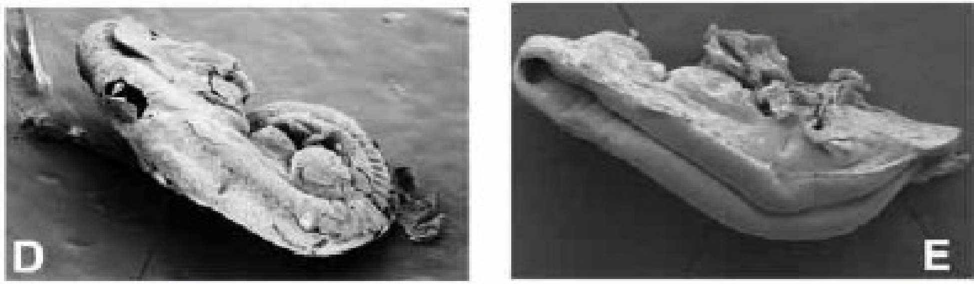
Důsledky narušené konvergentní extenze (CE)



Exencephaly

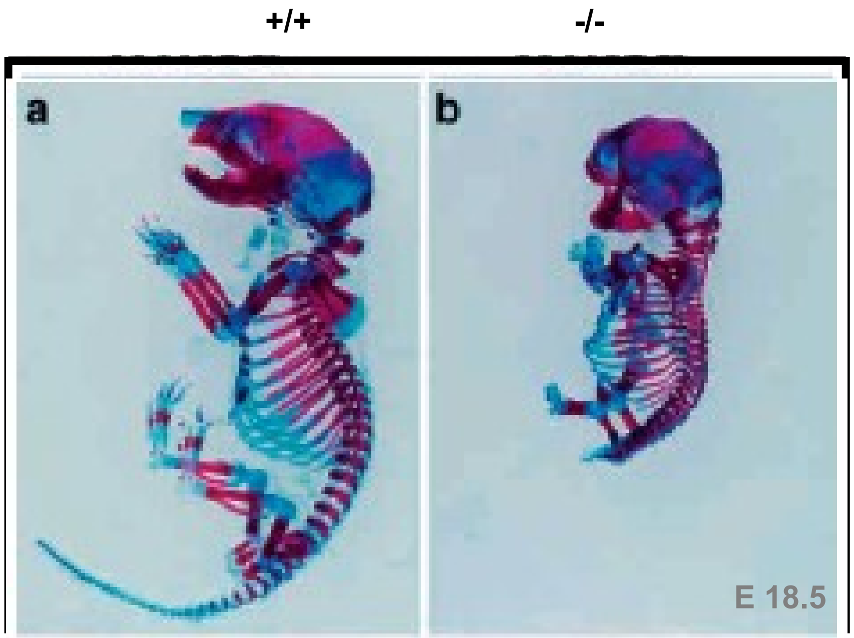


Open neural tube

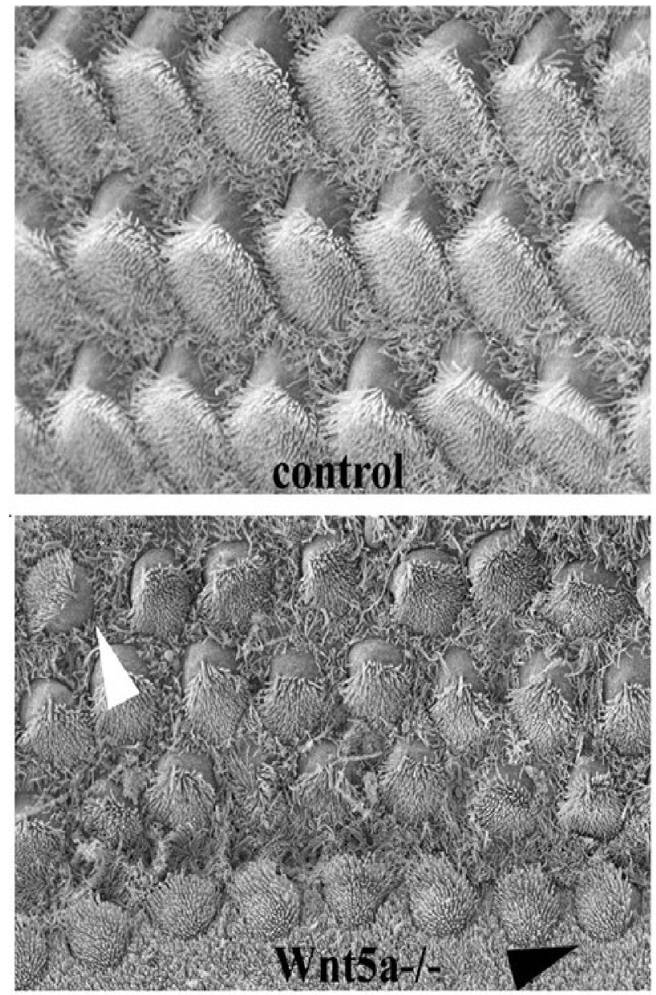


Hamblet et al., 2002, Development

Known Wnt5a knockout phenotypes

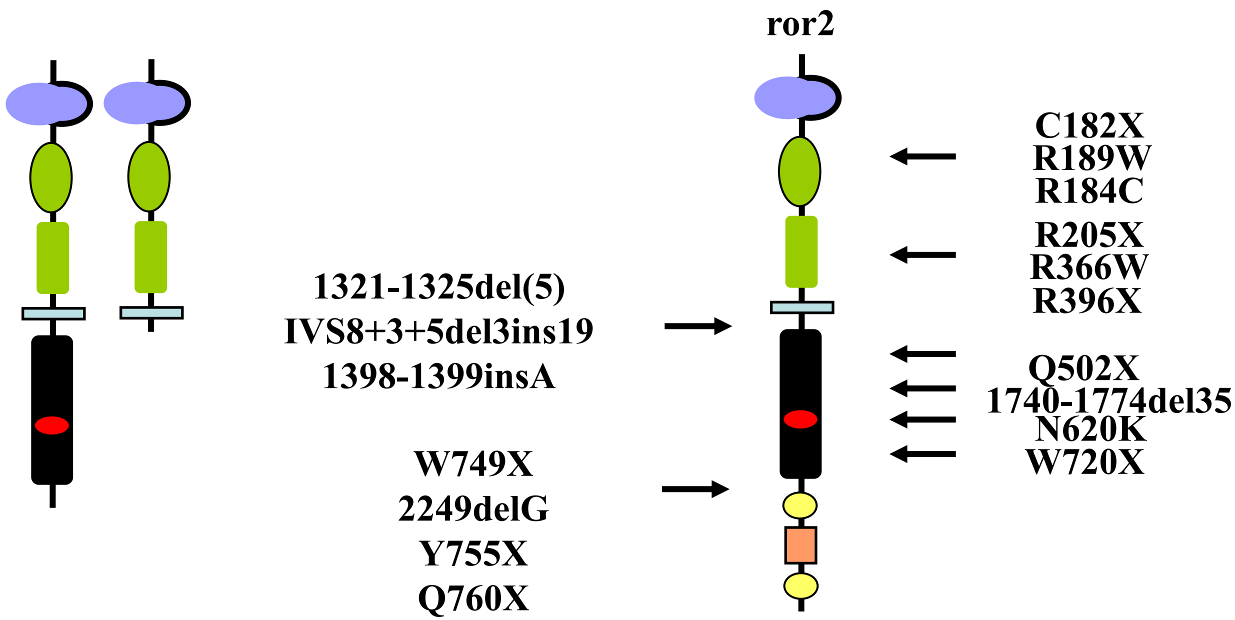


Yamaguchi et al., 1999



Qian et al, 2007

Mutations in *Ror2* cause dominant brachydactyly type B (BDB) and recessive robinow syndrome (RRS)

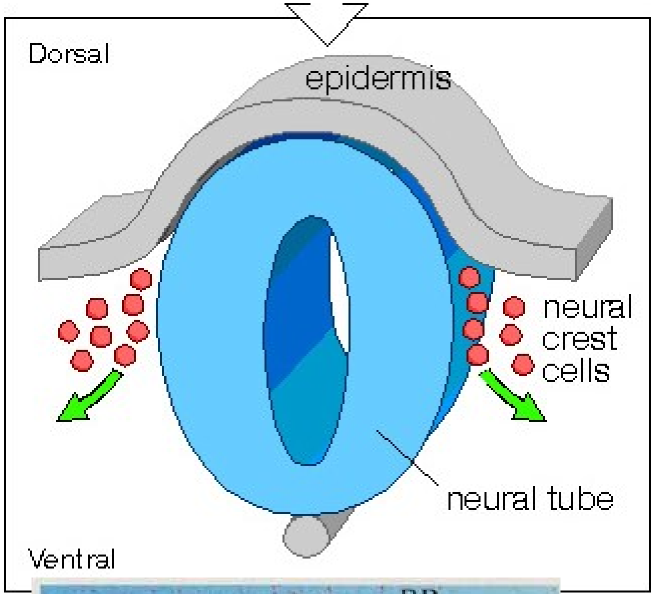


BDB

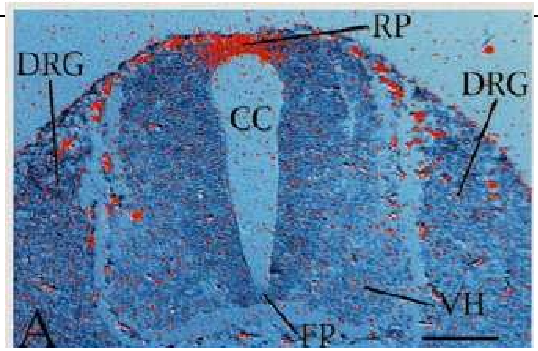
RRS

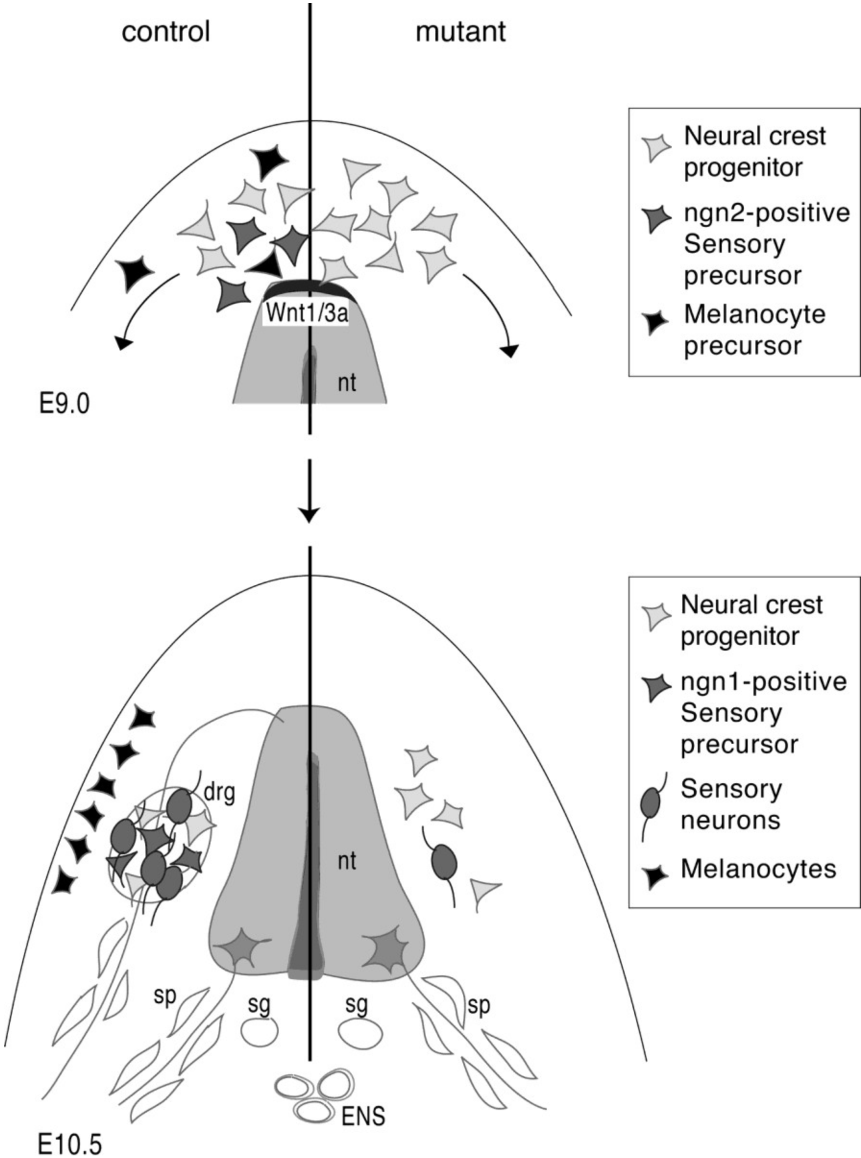
Kanonické a nekanonické Wnt signálování často regulují odlišné části téhož vývojového procesu.

I. Vývoj neurální lišty:



Wnt-3a

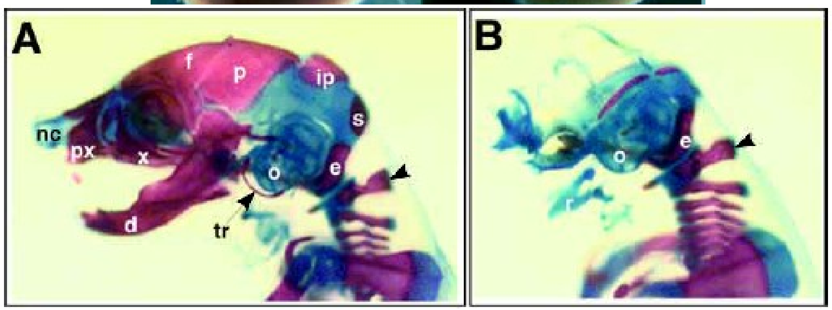


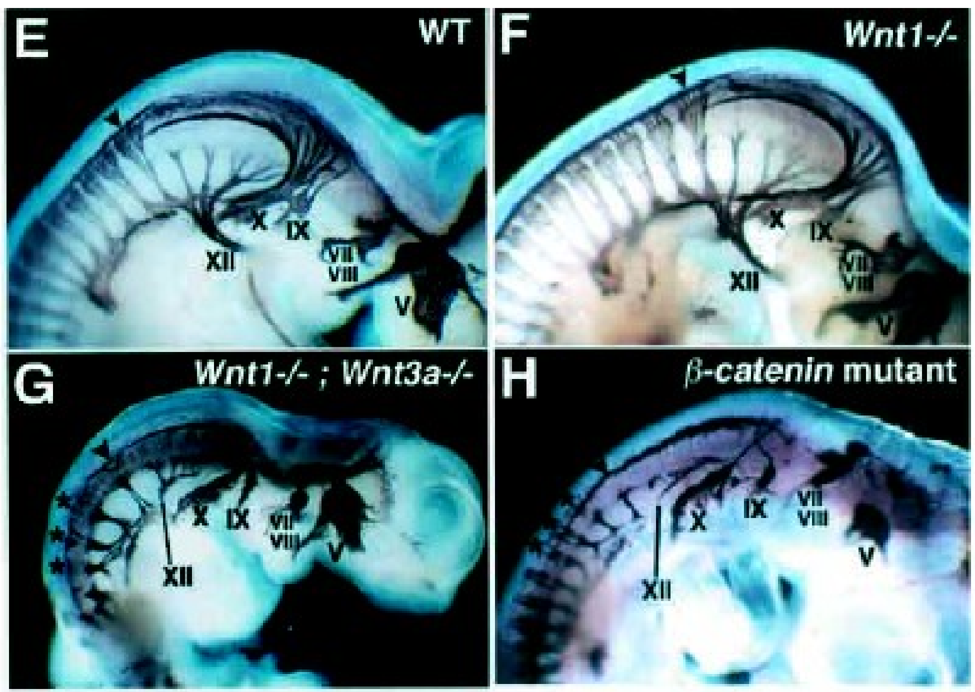


- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, srdce a dalších

Hari, L. et al. J. Cell Biol. 2002;159:867-880

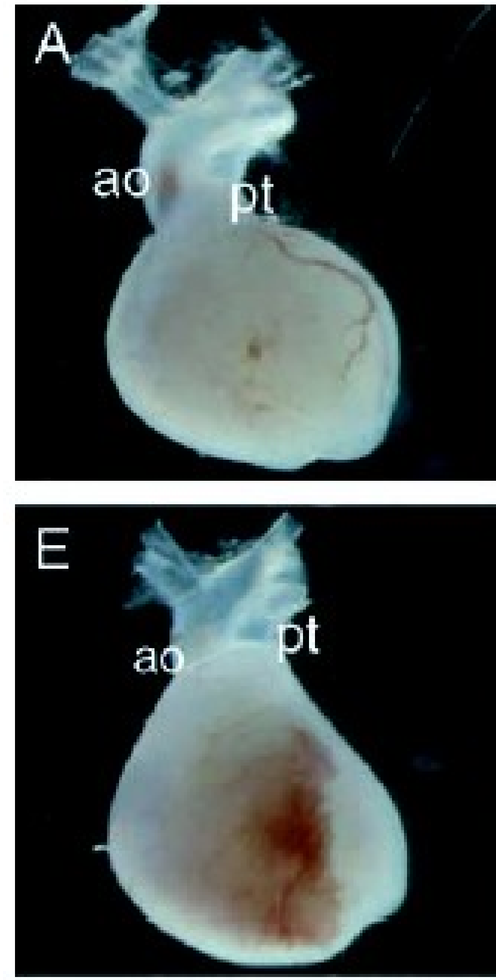
Wnt1/3a DKO





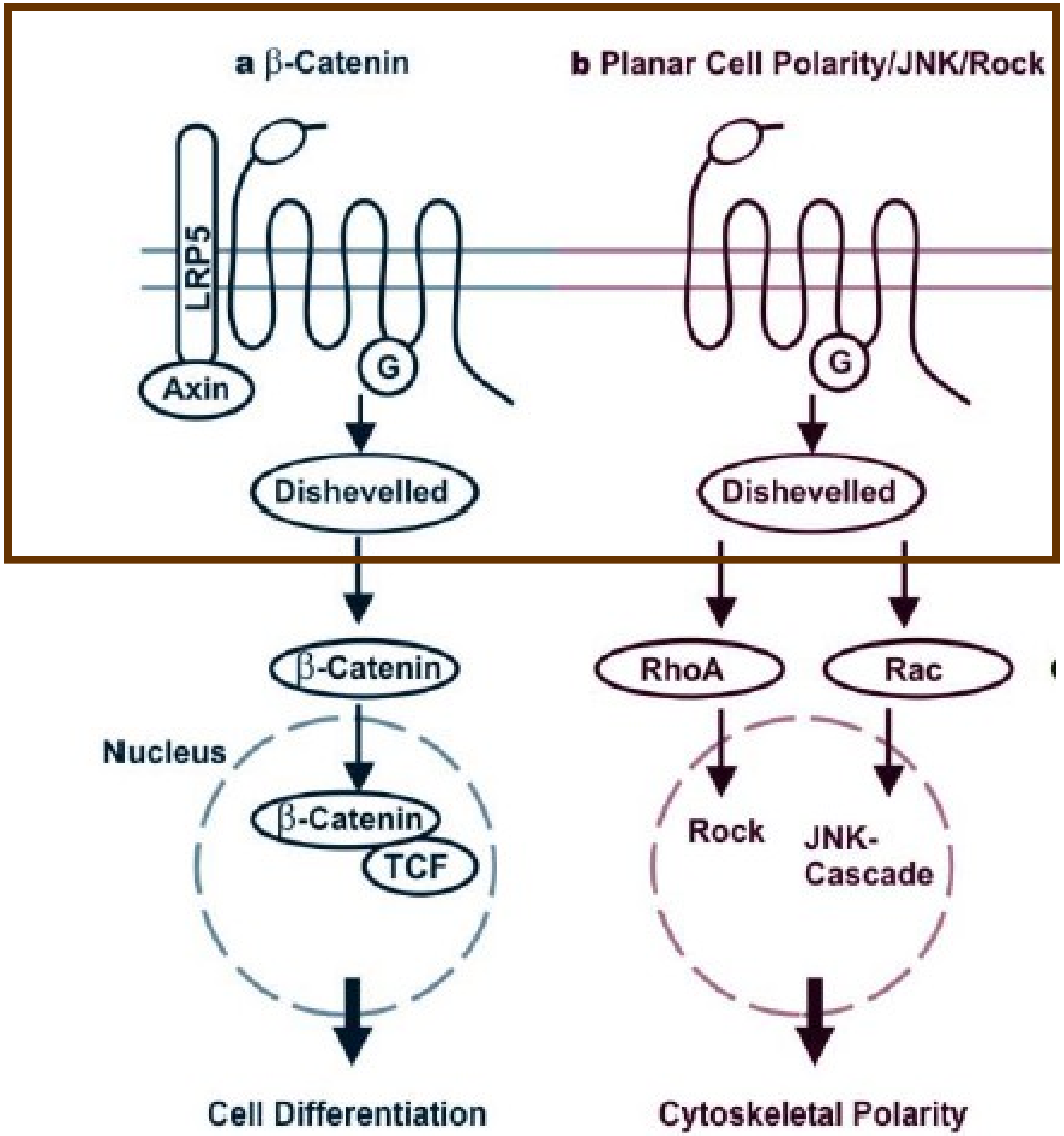
Heart outflow tract development

Wnt5a KO



Henderson DJ et al., 2006, TrendsCard. Res.

Fundamental question of Wnt signalling: How the specificity is achieved?



Vincan, 2004, Frontiers in Bioscience